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# SWEET CLOVER IN RELATION TO THE ACCUMULATION, LOSS AND CONSERVATION OF NITRATES IN SOIL

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The value of biennial white sweet clover when plowed under green, was emphasized by the authors in earlier publications (6, 7).

Maynard (1) made a study of the decomposition of this same variety under greenhouse conditions and reported that the three to four months' growth decayed very rapidly when used as a green manure. McBeth (2) tested green sweet clover in comparison with green oats, green barley, and green alfalfa and found the sweet clover easily the leader in nitrate production. In field trials with various legumes and non-legumes Mertz (3) found the annual sweet clover (*Melilotus indica*) the most promising winter green manure for southern California. The value of *Melilotus alba* for use as a green manure and for summer pasture was emphasized by Metzger (4) who recommended plowing it under when 8 to 10 inches high; and still more recently its importance has been pointed out by Moore and Graber (5) and by Fraser (5). Since the completion of this work Willard (8) has made a study of the yield and nitrogen content of white sweet clover at different dates. His results are in agreement with those already reported by the authors (7) and those occurring in this paper. He advises plowing between April 15 and May 10. On May 10 he found as much nitrogen as was found even as late as August 8. The weights of stubble and roots and the final stand per acre are reported by him.

Further studies were conducted by the authors on nitrification of the sweet clover as influenced by fall and spring plowing, spring plowing at different dates, and summer plowing of the crop the second year. Losses resulting from the last named practice and means of preventing it, and the rôle sweet clover plays in conserving soil nitrates during the fall, winter, and spring were also a part of this investigation. Important factors influencing the accumulation and the rate of production were next taken up from practical standpoints in the field. Attention was directed toward accumulations in the absence of succeeding crops with the purpose of studying nitrate conservation. The pro-

<sup>1</sup> The authors wish to express their appreciation of the excellent cooperation afforded by the experiment field staff of the agronomy department of the University of Illinois during the progress of this work.

The senior author is at present connected with the University of Wisconsin, and the junior author is on the Ohio State University staff.

tective action of sweet clover on soil, especially on the nitrates, was made a special study on experiment fields because the conservation of nitrates is often of equal importance to their accumulation.

Nitrate production in a soil together with nitrate utilization is only one factor concerned with crop production and, although a large factor in crop yields, is not necessarily related to crop yields unless optimal amounts of all other elements are present in optimal forms at all times. The highest nitrate producing plots can not give the largest yields if phosphorus, or any other factor of whatever kind, is in any manner limiting production.

Nitrogen must needs be studied as a single factor concerned with crop production, until studies of other elements meet the progress made on this element.

The studies that follow are concerned with practical field problems of controlling nitrate accumulation, conserving unused nitrates, and protecting the soil from losses, as related to the growth and plowing under of biennial white sweet clover (*Melilotus alba*).

#### NITRIFICATION OF FALL- VERSUS SPRING-PLOWED SWEET CLOVER, JOLIET EXPERIMENT FIELD—1921-1922

In order to study the effect of fall plowing compared with spring plowing of sweet clover, on its value for nitrate production for corn, Series 300 of the Joliet Field was divided, and the east half plowed in November and the west half in May.

Excellent plowing was accomplished in the fall, cutting the roots off completely at about seven inches, and turning the tops under properly. Samples were taken on both halves of the plots on November 25.

The plots selected for study were as follows:

Plot 305—no treatment.

Plot 307—limestone and sweet clover.

Plot 308—limestone, phosphorus, and sweet clover.

Plot 309—limestone, phosphorus, potassium, and sweet clover.

The sweet clover was in excellent condition on plot 309, good on 308, and fair on 307. In the early spring, the fall-plowed sweet clover began to grow, sending out many shoots from the crown of the old root and developing new feeding roots from the old root stock. A severe winter had not killed it, and it grew so fast that it made a fair stand. It was double-disked, but still it grew and finally it was plowed again in the spring about May 7 at the same time the west half was plowed. This experience with fall plowing of biennial sweet clover was not encouraging.

On May 5, before the spring plowing, samples were taken. The unplowed sweet clover was 14 inches high on plot 309 West, 12 inches on plot 308 West, and about 8 inches in height and irregular on plot 307 West. Spring rains kept the nitrates low up to May 5.

On July 6, the corn crop on this series showed the following differences in height:

<i>Plot</i>	<i>Height in inches</i>
305-E.....	17
305-W.....	20
307-E.....	18
307-W.....	32
308-E.....	24
308-W.....	36
309-E.....	30
309-W.....	40

The differences between the east and west halves were evident a considerable distance from the field until the corn became mature.

The reason for the difference in favor of the spring plowing was not easy to determine. In table 1 are given the nitrate results on the east and west halves.

TABLE 1

*Nitrate nitrogen in soil on Joliet Field, fall of 1921 and season of 1922*

[Pounds per acre in 2 million pounds of surface soil (about 0-6½ inches) water-free basis]

PLOT	TREATMENT	PLOWED	NITRATE NITROGEN ON DATE OF SAMPLING				
			November 25	May 5	June 7	July 6	August 18
305-E	0	Fall	16.21	19.41	29.13	32.82	56.18
305-W	0	Spring	15.22	15.50	24.91	23.11	29.74
307-E	L sweet clover	Fall	14.04	17.62	51.23	58.31	187.90
307-W	L sweet clover	Spring	12.80	19.78	59.06	61.21	113.00
308-E	LP sweet clover	Fall	13.76	15.48	91.47	70.76	83.32
308-W	LP sweet clover	Spring	15.82	5.91	97.24	63.76	67.36
309-E	LPK sweet clover	Fall	17.67	20.42	65.95	60.94	83.51
309-W	LPK sweet clover	Spring	17.04	7.48	89.85	74.10	158.38
309-E	LPK sweet clover	Fall	Subsoil*	29.52	50.98	37.97	
309-W	LPK sweet clover	Spring	Subsoil*	11.00	51.90	64.32	

\* In 6 million pounds of subsoil.

The nitrate content in the fall on November 25 was low in the surface soil and showed only slight differences between the east and west sides. These results show that the nitrate content of the surface soil had been reduced by a nearly uniform amount as a result of the 11 inches of rain from September to December. Although the checks showed 16.21 and 15.22 pounds in the surface soil, it must be remembered that the treated plots contained about the same amount of nitrate nitrogen, and in addition at least 100 pounds of nitrogen in the sweet clover crop whether plowed or unplowed. This important difference must not be overlooked in comparing the plots. The nitrogen in the crop is saved for another year. In the spring after 12.55 inches of rain, and with the growth of the crop on the treated plots, the nitrate was reduced on the 309 and 308 plots to very low figures. On plot 307 where the poorest

sweet clover was growing, an increase occurred. The checks also increased slightly. On plots 308 and 309 East and West, the nitrate results followed the corresponding sweet clover growths. The usual increases in nitrate content occurred after plowing and with the coming of increased temperatures. The treated plots showed much the highest nitrates at all times. The phosphorus and potassium plots, 308 and 309, in spite of the fact that they were supporting better corn, showed the highest nitrate content during the important feeding period for the corn crop.

The nitrate content of the subsoil of 309 East and 309 West showed the sweet clover had reduced it by its spring growth on the west side. After plowing, an increase in nitrate occurred in the subsoil. The differences in nitrate content between the fall plowing on the east side and the spring plowing on the west side, are not significant except on plot 309. The largest growth of sweet clover was plowed under on 309 West and here the largest nitrate occurred. The fact that all the nitrate figures are so far in excess of the other factors of growth shows that nitrogen was not limiting the growth of the corn.

The physical condition of the soil on the east side was very bad, whereas it was ideal on the west side. The spring disking and replowing of the east side gave a cloddy formation that endured all through the season. The large differences in the condition of the corn on the two halves may have been due to the poor physical condition of the east half. It is evident that the fall growth of sweet clover combined with its spring growth, in spite of twice double-disking on the east halves, furnished nitrates enough for about twice the crop obtained. The corn yields on the east and west halves did not differ by reliable differences, being only 1.8 bushels in favor of the east side on 308 and 1.4 bushels better on the west side of 309.

The yields on this series indicate clearly that phosphorus and potassium are not present in sufficient amounts in an available form to give as high corn yields as do nitrogen and limestone.

Such nitrate data are encouraging from the standpoint of the nitrogen problem, and clearly indicate, as has been shown (6, 7), that nitrification is ahead of crop requirements where proper soil treatment is applied, and where sweet clover is used repeatedly as a green manure in a 4-year rotation.

The nitrate results for August 18 are very high. These high amounts of nitrate in the surface of the soil are credited to a rise of nitrate from below that had not been lost from the subsoil, but which may be lost in the fall or the winter. The surface soil contained only 8.5 to 11.85 per cent of moisture on August 18. These percentages will not support nitrification in this soil. Similar nitrate concentrations in the surface will be presented in the data from other fields. This upward movement is an important consideration, especially at this time of year. Its recognition affords an opportunity to save the nitrate and thus to avoid undue losses.

Fall plowing on this field furnished more nitrate than was needed for one large corn crop. This makes desirable the finding of a satisfactory method

of fall plowing, as in some rotations the amount of nitrates here produced would be ample. This study indicates that spring plowing of green sweet clover is better than fall plowing, when all conditions such as nitrate production, physical condition, and lessened mechanical labor are considered.

NITRIFICATION OF SWEET CLOVER SPRING PLOWED AT DIFFERENT DATES,  
HARTSBURG AND TOLEDO EXPERIMENT FIELDS—1921

The effect of varying the date of plowing, on the recognized value of green sweet clover as a nitrate producer was studied in 1921 on the Hartsburg Experiment Field, in Logan County, and on the Toledo Experiment Field, in Cumberland County.

*Hartsburg Experiment Field*

The soil of the Hartsburg Field is of a heavy type, being classified as a black clay loam. Sweet clover will make a limited growth on the unlimed plot on this field.

The east side of series 300 was plowed on May 4 and the west side on May 13. Samples of the sweet clover were taken for analysis to determine the rate of nitrogen gain per acre. Unfortunately for this study some of the sweet clover was frozen three and some four times. The green weights and water-free weights of the crop at the time of plowing were determined and are reported in table 2.

The nitrate determinations in the surface soil are also arranged in table 2. The first samples were taken May 3. Some variations are evident between the east and west sides, especially on the treated plots. That the sweet clover stand was irregular was probably a contributing cause of the variations, as they are confined to given plots and do not carry across plot lines. The treated plots on which sweet clover had grown very well in the fall and only fairly in the spring, contained more nitrates than the checks where a poor growth of sweet clover was present.

As anticipated, the nitrates increased more rapidly on the east half after plowing, than on the unplowed west half, up to May 17. Both halves, however, increased; even the west side almost doubled in nitrate content. The cold weather apparently affected the growth of the sweet clover relatively more than it reduced the activity of the nitrifying bacteria. After some heavy rains the nitrate was reduced materially on all plots except 309, where the growth of sweet clover had been best. On June 22, the later plowing on the west sides contained much the larger amounts of nitrate, except on plot 308, both sides of which were, however, very high. Nitrates continued to accumulate on the east side although to a smaller extent. From July 1 to 15, conditions were highly favorable for the rise of nitrates from below through evaporation, although some was probably produced as the moisture did not fall to a prohibitive point on this field as it did on the Joliet Field. The large quantities

TABLE 2  
*Nitrate nitrogen in soil growing corn in 1921—Hartsburg Field sweet clover plowed May 4 and May 13*  
 [Per acre in 2 million pounds of surface soil (about 0-6½ inches) water-free basis]

PLOT	TREATMENT	FLOWED	WEIGHT OF SWEET CLOVER (TOPS ONLY)		NITRATE NITROGEN IN SOIL ON DATE OF SAMPLING									
			Green	Water-free										
			tons	tons	May 3	May 11	June 8	June 22	July 1	July 15	July 29	August 24	September 30	
305-E	0	May 4			15.7	61.9	30.7	29.0	35.9	78.0	52.7	28.54	26.1	lbs.
305-W	0	May 13			17.6	56.9	26.6	66.3	34.8	71.9	57.0	28.5	20.0	lbs.
306-E	R sweet clover	May 4	4.69	0.837	21.5	84.9	38.6	34.7	61.5	101.5	81.0	49.7	42.8	
306-W	R sweet clover	May 13			27.7	57.0	28.7	69.0	55.0	81.4	72.8	52.4	41.6	
307-E	RL sweet clover	May 4	4.30	0.731	25.2	88.7	34.4	53.0	90.6	78.7	75.0	46.1	28.9	
307-W	RL sweet clover	May 13			31.3	67.5	33.4	65.3	81.4	73.8	65.5	45.4	22.0	
308-E	RLP sweet clover	May 4	3.77	0.674	37.3	80.5	45.4	99.4	102.0	108.5	104.5	44.9	29.4	
308-W	RLP sweet clover	May 13			33.1	60.2	39.4	81.4	84.1	103.4	97.2	41.7	30.2	
309-E	RLPK sweet clover	May 4	5.73	1.010	33.2	73.2	62.6	83.1	81.5	92.0	75.1	36.1	30.5	
309-W	RLPK sweet clover	May 13	6.36	1.280	37.7	69.6	63.9	112.5	75.8	95.7	72.7	32.4	26.6	
310-E	0	May 4			17.6	58.4	26.0	34.6	47.3	56.1	52.1	20.0	28.2	
310-W	0	May 13			19.7	57.1	22.1	24.1	48.5	47.8	35.5	18.1	26.1	

Plots 306, 307 and 308-W killed by frost, no samples taken.

of nitrate found are indicative of the success attained in its production by proper soil treatment, including the use of green sweet clover. A corn crop of fifty to sixty-three bushels was being produced and the figures show that nitrate was not a limiting factor in production on this field. The presence of such large amounts of nitrate nitrogen during the critical feeding period, is proof that the production phase of the nitrogen problem is satisfactorily solved for such soils, until such a time as much larger crops are possible.

During August and September, as far as the surface soil is concerned heavy rainfall seriously reduced the nitrate content which fell from as high as 108 pounds to as low as 29. If this difference of 79 pounds was permanently lost, it means a serious depletion of the most expensive element, and its conservation by crop growth, by bacterial action, or by both, is highly desirable as the next important step in the solution of the nitrogen problem.

The average nitrate contents of the treated and untreated plots are given in table 3. The differences are interesting, as the checks are not acid, which

TABLE 3

*Average of nitrate nitrogen on treated and untreated plots on Hartsburg Field, 1921*

[Pounds per acre in 2 million pounds of surface soil (about 0-6 $\frac{1}{2}$  inches) water-free basis]

PLOT	NITRATE NITROGEN AVERAGES ON DATES OF SAMPLING								
	May 3	May 17	June 8	June 22	July 1	July 15	July 29	August 24	September 30
Treated . . . . .	30.90	72.70	43.30	74.80	78.99	91.88	80.45	43.57	31.50
Untreated . . . . .	17.65	58.58	26.35	38.65	41.60	63.45	49.40	23.78	25.08
Increase for treatment . . . . .	13.25	14.12	16.95	36.15	37.39	28.43	31.05	19.79	6.42

means that the increases found are largely due to the sweet clover and that they have occurred on a type of soil regarded as highly productive before sweet clover was used on it.

There was no important advantage, from a nitrogen standpoint, in either the May 4 or May 17 plowing dates, as both produced excessive nitrates for the critical feeding period. The organic matter and the other elements of plant-food contained in the sweet clover would be increased by allowing the crop to remain as long as possible before plowing.

### *Toledo Experiment Field*

The Toledo Experiment Field, in Cumberland County,\* in southeastern Illinois, is located on gray silt loam underlain with tight clay. This type of soil without treatment produces poor yields.

The south half of series 300, was plowed May 1, and the north half, May 16. Sweet clover samples were taken and the data on green and dry weights



TABLE 4  
*Nitrate nitrogen in soil growing corn in 1921 Toledo Field sweet clover plowed May 1 and 16*  
 [Per acre in 2 million pounds of surface soil (0-6½ inches) water-free basis]

PLOT	TREATMENT	PLOWED	WEIGHT OF SWEET CLOVER (TONS ONLY)		NITRATE NITROGEN IN SOIL ON DATE OF SAMPLING									
			Green	Water-free										
			tons	tons	April 22	May 4	May 14	June 7	June 23	June 30	July 14	July 27	August 25	September 29
305-S	0	May 1			15.4	20.2	35.9	16.4	17.1	31.9	65.1	40.4	31.9	24.0
305-N	0	May 16			7.6	11.6	34.5	15.8	20.5	34.9	63.8	39.4	22.0	26.6
306-S	R	May 1			23.4	23.2	34.3	19.3	19.6	30.7	64.9	43.2	21.5	23.5
306-N	R	May 16			7.7	20.9	36.5	21.9	19.5	28.2	69.6	39.9	18.6	23.2
307-S	RL sweet clover	May 1	2.83	0.560	23.5	22.5	68.9	43.4	26.2	63.3	103.0	82.3	43.2	44.8
307-N	RL sweet clover	May 16	5.80	1.052	22.9	25.2	55.4	44.5	25.4	53.7	114.2	77.8	34.5	35.8
308-S	RLP sweet clover	May 1	2.10	0.421	23.1	19.3	66.7	46.6	57.7	57.2	100.4	74.9	38.1	40.1
308-N	RLP sweet clover	May 16	5.27	0.923	15.4	20.7	49.3	43.1	50.7	53.8	90.9	84.5	33.0	33.5
309-S	RLPK sweet clover	May 1	4.23	0.794	23.0	19.6	56.9	58.0	65.0	76.6	107.5	105.0	46.9	35.6
309-N	RLPK sweet clover	May 16	11.78	1.840	30.6	22.8	40.0	60.4	24.5	100.6	113.2	89.4	36.6	34.5
310-S	0	May 1			23.1	9.7	30.3	23.8	17.7	30.9	64.7	53.5	32.0	23.2
310-N	0	May 16			7.7	11.7	35.0	20.5	19.7	29.4	67.3	50.0	17.8	24.8

are found in table 4. The dry weight of the sweet clover tops doubled in the 15-day interval.

The nitrate content of the south side was greater on May 14, thirteen days after plowing. The north side showed less regularity in surpassing the south side, even as late as July 14. Ample nitrate was produced on both sides of the plots. A very poor crop was present on the checks which accounts in part for their high nitrate content, but most of the nitrate accumulation on the checks is ascribed to the rest period which this series enjoyed through three successive crop failures. On this field, the different dates of plowing did not show sufficient difference in the nitrate content to affect the crop. A large surplus was present during the critical feeding period.

The excessive amounts in the surface soil were derived from the lower layers and not from the amount manufactured in the surface at that time, as the moisture was very low during July, ranging from 6.4 per cent on plot 309

TABLE 5

*Average of nitrate nitrogen of treated and untreated soil on Toledo Field, 1921*

[Pounds per acre in 2 million pounds of surface soil (about 0-6 $\frac{1}{2}$  inches) water-free basis]

PLOT	NITRATE NITROGEN ON DATE OF SAMPLING									
	April 22	May 4	May 14	June 7	June 23	June 30	July 14	July 27	August 25	September 29
Treated .....	23.10	21.70	56.20	51.00	41.60	67.50	104.90	85.65	38.71	37.38
Untreated .....	14.15	16.20	34.40	19.60	19.00	31.00	65.90	44.40	23.95	24.21
Increase for treatment ....	8.95	5.50	21.80	31.40	22.60	36.50	39.00	41.25	14.76	13.17

to 11.6 per cent on the check plot 310. The rainfall was 1.92 inches for July, 5.94 inches for August, and 8.59 inches for September. The accumulation under excessively dry soil conditions is again explained by a rise of nitrates. The great losses from the surface soil due to rain, are again apparent. Plots 307, 308, and 309 averaged 104.8 pounds of nitrate nitrogen on July 14 and only 38.71 on August 25 and 37.36 pounds on September 29. A loss of about 67 pounds occurred during this period. On this type of soil with an impervious subsoil, the nitrate may not leach out, as this land is not tile drained, but it may be lost through denitrification when conditions of moisture and temperature are favorable. On properly tiled land a loss would result from leaching.

In table 5, the average nitrate nitrogen contents of the untreated and the treated plots are reported. Treated soil growing sweet clover furnished much larger amounts of nitrates (in spite of the fact that these plots were producing much larger crops) than the checks.

It is evident that on this field, as on the others studied, the nitrogen requirements have been much more successfully met than the other factors concerned

in production. Lack of sufficient moisture, injury due to hot winds, lack of available phosphorus, or lack of some other element than nitrogen, must be regarded as the cause of limited crop growth on this field.

Data on the height, green weight, percentage of nitrogen, and amount of nitrogen in the tops per acre, are given in table 6. The increases in nitrogen in the tops per acre are apparent on the various dates of sampling. The crop contains over 80 pounds of nitrogen per ton on both these fields, except on May 14 and 17. Delaying the plowing, greatly increases the weight of organic matter and the nitrogen per acre in the tops. These figures would appear to represent the minimum, as the exceptionally cold weather accom-

TABLE 6  
*Weights and nitrogen content of sweet clover tops of spring growth, 1921*  
(Acre basis)

FIELD	PLOT	DATE SAMPLE WAS TAKEN	HEIGHT	GREEN WEIGHT	WATER	WEIGHT OF WATER	WATER-FREE MATERIAL		WEIGHT OF NITRO- GEN IN TOPS
							Weight	Nitrogen	
			<i>inches</i>	<i>tons</i>	<i>per cent</i>	<i>tons</i>	<i>tons</i>	<i>per cent</i>	<i>lbs.</i>
Toledo.....	307-S	April 22	6	2.420	85.68	2.073	0.346	4.26	29.5
	308-S	22	5	1.960	85.20	1.670	0.290	4.44	25.7
	309-S	22	8	4.840	87.33	4.227	0.613	4.34	53.2
	307-S	May 4	8	2.831	80.21	2.271	0.560	4.44	49.7
	308-S	4	7	2.105	80.00	1.684	0.421	4.30	35.4
	309-S	4	13	4.235	81.23	3.440	0.794	4.22	67.0
	307-N	14	18	5.808	81.87	4.755	1.052	3.74	78.7
	308-N	14	17	5.275	82.19	4.352	0.923	3.60	67.4
	309-N	14	24	11.785	84.33	9.939	1.845	3.74	138.0
Hartsburg...	306-E	May 3	6	4.694	82.17	3.857	0.837	4.42	73.9
	307-E	3	6	4.307	83.02	3.576	0.731	4.44	64.9
	308-E	3	6	3.777	82.14	3.103	0.674	4.50	60.7
	309-E	3	8	5.735	82.32	4.721	1.013	4.22	85.5
	309-W	17	20	6.364	79.76	5.076	1.288	3.88	100.0

panied by repeated freezes delayed the growth of the sweet clover more than in any other season noted.

The need for delaying the plowing of sweet clover on this type of soil is most urgent in the initial use of the crop. After it has been grown two or more times on the same soil, it may be plowed earlier without sufficiently reducing the accumulation of nitrate to limit the corn crop.

#### NITRIFICATION OF SUMMER PLOWED SWEET CLOVER, BLOOMINGTON, ILLINOIS

Through the coöperation of the Bloomington Canning Company and the McLean County Farm Bureau, an opportunity to study nitrate accumulation and losses was available where second year sweet clover was plowed

under. The soil of this field is a brown silt loam and had been limed. The sweet clover was about 6 feet high when plowing began about July 22. The tops were still green and seed had not yet formed. There were 100 acres of the section in second year sweet clover.

The question arose as to the loss of nitrates that might result from plowing while the crop was still green and with about ten months intervening before the sweet corn would be planted. It was suggested that oats and rye be seeded on adjacent plots and these together with a fallow check would be sampled for nitrate accumulation and losses. The purpose of seeding these

TABLE 7  
*Nitrate nitrogen in soil at Bloomington, Illinois: Field of Bloomington Canning Company.*  
*Plowed under the Last of July, 1921*  
(Water-free basis)

TREATMENT	POUNDS OF NITRATE NITROGEN IN SURFACE, SUBSURFACE, AND SUBSOIL			
	October 1 1921	November 3 1921	May 4 1922	July 5 1922
<i>Fallow:</i>				
Surface, 0-7 inches.....	78.5	103.3	7.8	40.9
Subsurface, 7-20 inches.....	83.7	107.2	29.2	60.5
Subsoil, 20-40 inches.....	60.2	92.5	56.3	77.9
	222.4	303.0	93.3	179.3
<i>Rye:</i>				
Surface, 0-7 inches.....	64.6	34.5	9.7	51.5
Subsurface, 7-20 inches.....	107.2	94.0	34.7	61.3
Subsoil, 20-40 inches.....	99.0	85.7	62.4	82.2
	270.8	214.2	106.8	195.0
Subsoil, 40-80 inches.....	182.4	182.2	127.8	160.3
<i>Oats:</i>				
Surface, 0-7 inches.....	56.2	29.5	27.9	50.3
Subsurface, 7-20 inches.....	84.6	63.1	48.9	46.6
Subsoil, 20-40 inches.....	85.2	91.2	69.9	50.4
	226.0	183.8	146.7	147.3

crops at this time was to determine their value for conserving the nitrates that would be produced from the green sweet clover. Plots of about one-fourth acre in area were arranged at the northeast corner of the field. Samples were taken soon after plowing and on August 25, about one month later but before the small grains were seeded. The surface soil contained 77.8 pounds of nitrate nitrogen per acre on July 29 and 49.1 on August 25. There was 10.44 inches of rain in August which accounts for the reduction noted. Beginning October 1, samples were taken of the three plots at three depths and a few samples were taken as deep as 80 inches.

From table 7 it is evident that the sweet clover increased the nitrate con-

tent of the fallow soil, as on November 3 about 103.3 pounds was found in the surface, 107.2 in the subsurface, and 92.5 in the subsoil or a total of 303.0 pounds in 40 inches. This was from two to three times as much as the highest producing experiment fields sampled at the same time and in the same manner. The much lower nitrate content of the oat and rye plots is evident in the surface and subsurface. The amount of nitrates in the subsoil was about the same as that of the fallow, which may be accounted for by the heavy rain which washed the soil equally on all plots before the oats and rye grew sufficiently to use nitrates and to offer protection against its descent. The oats attained a height of 14 inches and covered the ground. The rye was a thin stand, but stooled out to make a fair stand about 4 inches high by November 3. There was a difference of 88.8 pounds between the fallow and the rye plots and 119.2 pounds between the fallow and the oats plots on November 3. Such differences are not to be accounted for only in the crops. The actual conservation of nitrogen, as shown by the oat plot, was probably greater than shown by the figures, because on the fallow plot the initial nitrification was more complete, whereas on the other plots it was delayed at certain periods

TABLE 8

*Distribution of nitrate by inches sweet clover field of Bloomington Canning Company, 1921*  
(Pounds per inch)

LAYERS	FALLOW	RYE	OATS
Surface, 0-7 inches. ....	14.7	4.9	4.2
Subsurface, 7-20 inches. ....	8.2	7.2	4.8
Subsoil, 20-40 inches. ....	4.6	4.3	4.6
Subsoil, 40-80 inches. ....	4.6	4.6	

by the presence of the crops. In the spring the oats plot, as expected, contained the highest nitrate in the surface soil. The rye grew until disked in. The nitrate on this plot was only 9 pounds. The fallow plot suffered a loss from leaching and therefore contained only 7 pounds. In July when the sweet corn was growing rapidly, there was an excess of nitrate on all plots.

The oat plot was the most efficient and the most economical in the utilization of the nitrate. It contained about 50 pounds more than the fallow on May 4. July 5, it was the lowest of the three plots, which as in the previous year, would mean smaller losses.

Soil that possesses the least nitrate in the fall, sufficient preceding and an excess during the critical feeding period of the crop, and the least after meeting this feeding period is the most desirable for the corn crop. Such a soil must possess the potential capacity to produce ample nitrate, as did this soil.

The distribution of the nitrate nitrogen by inches in the various layers, as seen in table 8, shows readily the efficiency of these crops in checking the downward movement of the nitrates in the subsurface where the effect would be most easily determined. The conservation of nitrates is also readily seen

from this table. With a growing crop on the surface soil, a conversion is taking place at the same time that a reduced nitrification occurs, which means a supply of organic nitrogen for nitrification at a later period. Volunteer oats and other grains function in a limited degree in the manner indicated here. In cases where land is not to be used for growing other crops, allowing sweet clover to reach maturity will greatly increase the organic matter content of the soil and provide nitrate nitrogen for two corn crops. When the crop is left for seed the rate of nitrification is, however, advantageously delayed and fall and spring losses are reduced. The volume of organic matter is not so great when the crop is left for seed as when plowed under green at an earlier stage of maturity.

The above method of handling sweet clover is not so valuable as plowing it under green in the spring because a year is not lost in growing a money crop. The magnitude of the losses that may occur are evident from this study and are perhaps as large here as would be met under almost any other condition, because of the large interval occurring between plowing the green, rapidly decomposing crop and planting the succeeding crop, and of the excessive fall and spring rains of that period.

Sweet clover as a nitrate producer is further demonstrated in this investigation at Bloomington.

#### LOSS OF NITRATE ON ILLINOIS EXPERIMENT FIELDS, 1921-22

It was the purpose of this study to determine the amount of nitrate lost from the 40-inch layer of the soil during the winter and early spring and the effect of the treatments on reducing losses.

Plot 404, which receives manure, limestone, and phosphate; the adjacent check, and the nearest sweet clover plot receiving limestone and phosphate, were selected on eight fields in northern and central Illinois, and on five in southern Illinois.

Fall samples were collected in November and December. The Dixon, Mount Morris, La Moille, and Spring Valley samples were taken from under 2 to 4 inches of snow. The remainder of the samples from the northern and central fields were taken after continuous rains and with the soil very wet. The samples from the southern fields were taken after heavy rains.

In table 9, the pounds of nitrate per acre in surface, subsurface, and sub-soil in the fall and spring are reported. The differences represent the amounts lost. The totals are found in the last three columns at the right. Where a gain occurred a plus sign appears before the figures; all other figures are losses. Manure had been spread on all the northern fields; this would increase the losses found.

It should be understood that wide variations occur in the soil types among these fields and in most cases on a given field. The plots included in the study on the Dixon field extended over two phases of brown silt loams; on the Spring Valley field one phase of brown silt loam only entered into the

TABLE 9  
*Loss of nitrate nitrogen on Northern and Central Illinois Experiment Fields during the winter and spring of 1921-22*  
 (Pounds per acre)

FIELD	DATE	SURFACE			SUBSURFACE			SUBSOIL			TOTAL		
		404	405	408	404	405	408	404	405	408	404	405	408
Dixon.....	Fall	18.36	21.24	18.07	34.29	34.22	30.00	43.21	56.09	37.41	105.86	111.55	85.48
	Spring	17.17	4.62	16.23	24.28	20.68	29.62	33.90	25.53	39.40	75.35	50.83	85.25
	Loss	1.19	16.62	1.84	10.01	13.54	0.38	19.31	30.56	+1.99	30.51	60.72	0.23
Mount Morris.....	Fall	22.05	23.24	19.27	34.34	52.60	28.42	46.05	53.27	52.41	102.44	129.11	90.10
	Spring	10.56	15.84	12.75	29.32	23.58	29.79	29.64	65.63	53.38	69.52	106.05	95.92
	Loss	11.49	7.40	6.52	5.02	29.02	+1.37	16.41	+12.36	+10.97	32.92	23.06	+5.82
LaMoille.....	Fall	44.39	40.28	38.51	52.19	48.73	59.48	39.27	42.24	83.12	135.85	131.25	181.11
	Spring	11.98	8.01	20.84	19.06	19.22	19.04	22.54	22.68	28.18	53.58	49.91	68.06
	Loss	32.41	32.27	17.67	33.13	29.51	40.44	16.73	19.56	54.94	82.27	81.34	113.05
Spring Valley.....	Fall	14.58	15.41	18.38	35.58	26.39	30.07	39.39	37.20	46.73	89.55	79.00	95.18
	Spring	12.92	12.55	16.36	18.10	18.41	29.16	21.92	16.65	50.12	52.94	47.61	95.64
	Loss	1.66	2.86	2.02	17.48	7.98	0.91	17.47	20.55	+3.39	36.61	31.39	+0.46
Kewanee.....	Fall	24.24	28.13	29.49	46.73	56.60	46.51	51.28	68.31	53.99	122.25	153.04	129.99
	Spring	12.48	14.20	16.16	56.16	24.21	27.77	41.95	53.61	24.10	110.59	92.02	68.03
	Loss	11.76	13.93	13.33	+9.43	32.39	18.74	9.33	14.70	29.89	11.66	61.02	61.96
Aledo.....	Fall	22.45	19.34	18.70	37.37	29.64	30.51	34.04	31.55	31.20	93.86	80.53	80.41
	Spring	13.60	9.76	16.05	34.02	34.52	39.43	33.47	34.32*	45.95	81.09	78.60	101.40
	Loss	8.85	9.58	2.65	3.35	+4.88	+8.92	0.57	+2.77	+14.75	12.77	1.93	+20.99

Oquawka.....	Fall	11.45	9.68	15.57	24.55	19.64	26.21	37.29	43.97	34.34	73.23	73.29	76.12
	Spring	8.45	3.12	6.70	16.19	9.80	15.25	44.05	20.70	9.57	68.69	33.62	31.52
	Loss	3.00	6.56	8.87	8.36	9.84	10.96	+6.76	23.27	24.77	4.54	39.67	44.60
Carthage.....	Fall	12.07	10.97	18.29	29.33	25.26	25.15	31.83	28.94	28.80	73.23	65.17	72.24
	Spring	9.53	5.77	17.04	18.90	11.42	24.63	28.90	23.10	17.02	57.33	40.29	58.69
	Loss	2.54	5.20	1.25	10.43	13.84	0.52	2.93	5.84	11.78	15.90	24.88	13.55

\* One determination only.



plots; on the La Moille field a black silty clay loam, a black clay loam, and a brown silt loam entered in; on the Mount Morris field a light brown silt loam and a light brown silt loam shallow phase were included in the plots; on the Kewanee field all plots occur on the brown silt loam; a similar condition exists on the Aledo field; on the Carthage field a black silty clay loam on clay and a grayish brown silt loam on tight clay were the types on the plots selected. The Oquawka and Palestine fields are sands.

Toledo, Newton, and Oblong are gray silt loams on tight clay. West Salem is a yellow gray silt loam. These four southern fields and Palestine represent types of soil which respond in a large way to treatment. The sweet clover plots (numbers 408) of the northern and central groups, lost the smallest amount or gained on the Dixon, Mount Morris, Spring Valley, Aledo, and Carthage fields. The manure plots (numbers 404) lost the smallest amount on two fields of this group, Oquawka and Kewanee. The checks lost the largest amounts on Dixon, Kewanee, and Carthage. On the La Moille field, the check and manure plot lost about the same amount.

The loss of nitrate nitrogen irrespective of the crop on the sweet clover plots was 206; on the manure 227; and on the check 325 pounds per acre. The average reductions in loss on the northern and central fields were as follows:

	<i>pounds per acre less than check plots</i>
Sweet clover plots (408).....	119
Manure plots (404) .....	98

In addition to the reduction of losses, the sweet clover contained at least 100 pounds and probably 150 pounds of nitrogen, a part of which was originally nitrate that would otherwise have been lost.

The percentage loss in most cases was greatest on the check plots. Where the largest amounts were present, the loss was somewhat dependent upon the contour of the field, as on the La Moille field. On the Kewanee field, the fox-tail was an important factor in preventing much larger losses.

These studies were made during a season of disastrous rainfall—September and March were about four times the normal rainfall and April was only a little under March for most of the fields.

The outstanding value of sweet clover on these fields in reducing the losses of nitrate nitrogen by protecting the soil and as a conserver of nitrogen, needs no further comment.

On the southern fields, which contain much less nitrate under most conditions, the losses were small both actually and on a percentage basis. There is a possibility that early spring nitrification reduced some of the losses. That the manure plots gained on three fields, on which the sweet clover was growing, supports this view, for the growth of sweet clover would increase the apparent loss of nitrogen by conversion. When the large sweet clover crops on these fields, as on the others studied, are taken into consideration, the ap-

TABLE 10  
*Loss of nitrate nitrogen on Southern Illinois Experiment Fields during the winter and spring of 1921-22*  
 (Pounds per acre)

FIELD	DATE	SURFACE			SUBSURFACE			SUBSOIL			TOTAL		
		404	405	408	404	405	408	404	405	408	404	405	408
Toledo.....	Fall	11.64	11.71	11.30	17.13	18.92	19.43	25.44	22.57	22.50	54.21	53.20	52.33
	Spring	5.81	5.77	11.63	22.39	11.34	19.13	28.71	23.19	11.19	56.91	40.30	41.95
	Loss	5.83	5.94	+0.33	+5.26	7.58	0.30	+3.27	+0.62	11.31	+2.70	12.90	10.38
Newton.....	Fall	9.60	10.52	13.30	32.66	23.37	20.57	27.08	30.93	23.84	69.34	64.82	57.71
	Spring	9.05	5.65	5.57	41.33	18.90	7.36	26.35	26.97	20.89	76.73	51.52	33.82
	Loss	0.55	4.87	7.73	+8.67	4.47	13.21	0.73	3.96	2.95	+7.39	13.30	23.89
Oblong.....	Fall	12.80	11.74	12.57	23.22	19.04	18.00	31.32	19.85	37.89	67.34	50.63	68.64
	Spring	6.06	9.21	17.10	18.64	18.59	15.07	38.54	16.85	28.58	63.24	44.65	60.75
	Loss	6.74	2.53	+4.53	4.58	0.45	2.93	+7.22	3.00	9.31	4.10	5.98	7.89
Palestine.....	Fall	8.22	7.44	9.14	8.28	13.23	23.12	18.00	22.41	19.86	34.50	43.08	59.12
	Spring	2.56	9.09	14.59	18.26	29.45	18.28	13.08	15.75	21.95	33.90	54.29	54.82
	Loss	5.66	+1.65	+5.45	+9.98	+16.22	4.84	4.92	6.66	+2.09	0.60	+11.21	4.30
West Salem.....	Fall	10.04	11.80	16.52	18.61	29.54	20.57	22.05	21.53	27.50	50.70	62.87	64.59
	Spring	7.83	3.62	8.82	30.05	7.52	25.65	34.78	10.84	11.19	72.66	21.98	45.66
	Loss	2.21	8.18	7.70	+11.44	22.02	+5.08	+12.73	10.69	16.31	+21.96	40.89	18.93

parent losses become actual gains of about 100 pounds per acre for the sweet clover plots.

The manure plots gained 27.5 pounds per acre instead of losing 61.86 as the checks did. If the difference came from nitrification of the manure, it offsets the comparison.

This study was conducted during the wettest spring recorded for a great many years. In a normal fall, winter, and spring, greater differences would be found in favor of the sweet clover as a conserver.

#### SUMMARY

Nitrification of both fall and spring-plowed sweet clover proceeded rapidly and to such an extent on the Joliet Field as to furnish nitrate in excess of the requirements of a large corn crop. The spring-plowed area was in better physical condition and required less labor in preparation than the fall-plowed area. More organic matter was plowed under on the spring-plowed half, which is one of the most important considerations in the initial use of sweet clover. Fall plowing of sweet clover is frequently desirable, but until more information is available as to thorough methods of killing the crop, spring plowing should be the general practice.

The rate of nitrification of sweet clover plowed in the spring, at different dates prior to corn planting, coincided closely with the date of plowing, that is, early plowing gave higher nitrate at an earlier date than later plowing. At both Hartsburg and Toledo, all dates of plowing permitted a rapid nitrification and an accumulation sufficient to meet the needs of much larger crops than were produced. As sweet clover had been grown several times as a green manure on each of these series, a generally higher level of nitrate accumulation than in soils not so treated exists here, and this condition reduced the possibility of larger differences in nitrates resulting from the effect of prolonging the spring growth period.

The date of plowing in the spring should be decided according to the urgency of the need of the soil for active organic matter, as the condition of sweet clover during all the time available for spring plowing ensures its rapid nitrification. The sweet clover on light, sandy, and open textured soils and on those deficient in organic matter should be plowed as late as consistent with good soil preparation for the corn crop. Early plowing of the sweet clover on heavy types, clays, and silts, and on those soils where the crop has grown one or more times, will not materially affect corn production.

Summer-plowed green sweet clover nitrifies rapidly and large amounts of nitrate accumulate, as indicated by the results reported from the study at Bloomington. Large losses result if no protective crop is seeded. Oats and rye proved efficient in converting much nitrate into organic nitrogen and in reducing the amount formed, both of which reduced the losses. The oats were more valuable in reducing losses than the rye, because of their greater fall growth and the fact that they were incorporated with the soil in a dry condition instead of in the green condition.

The handling of sweet clover in this manner is not desirable except in special cases, for equal soil enrichment can be accomplished by using the crop as a green manure, without sacrificing a year to the growing of this crop.

Nitrates concentrate in the surface soil, rising from lower layers. The rise is proved by the fact that it occurred with the moisture content of the surface soils below the point of supporting nitrification. In many cases over 100 pounds of nitrate nitrogen was found in the surface, even in the presence of a crop of 50 to 65 bushels of corn, which was practically produced at this time. Such amounts of nitrate nitrogen should be converted by crops, or by bacteria in order to conserve them for use by succeeding crops. Under farm conditions, weeds in the corn, volunteer grains, and any crop growth on the land in late summer and fall serve to convert much nitrate. If a legume is used, the nitrogen so saved is rapidly and more completely nitrified the following year. Thus, if nitrates are not readily available, use a legume; if nitrates are present in ample amounts, use a grain crop for conserving them.

Two-year rotations of corn, wheat, and sweet clover, or corn, rye, and sweet clover, or corn, barley, and sweet clover seeding sweet clover in the grain, plowing it for corn, and seeding wheat or barley on the corn land, would be efficient from many standpoints especially that of the economical production, utilization, and conservation of nitrate nitrogen. Wheat following oats has been successful in general practice because the moisture, nitrates, and other food have been stored up in the surface during the summer for its fall growth. Rains in the summer do not usually cause serious leaching of plant-food, especially if the oat land is plowed early for wheat.

Studies on thirteen Illinois Experiment Fields during a most disastrous season of rainfall, demonstrated the importance of sweet clover in nitrogen economy. Less nitrate was lost and a large amounts was converted into organic nitrogen by the sweet clover. These results were obtained where sweet clover is grown as a green manure in a four-year rotation.

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# INFLUENCE OF FORM, SOIL-ZONE AND FINENESS OF LIME AND MAGNESIA INCORPORATIONS UPON OUTGO OF SULFATES AND NITRATES<sup>1</sup>

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Among the benefits attributed to the liming of soils is that of accelerating those biological processes which give increased amounts of soluble nitrogen and sulfur for plant growth. Added alkali-earth carbonates, or those derived from  $\text{CaO}$  or  $\text{Ca(OH)}_2$  additions, may be decomposed by two processes: They may be dissolved by the carbonated soil water, diffused, and fixed largely in silicate combinations, or they may be decomposed by direct action of biologically-induced nitric and sulfuric acids. The former process depends upon solution and movement of bicarbonates to the surfaces of the acidoids. The latter may be conceived as resulting from the localized generation of the biologically-induced acids and their diffusion to the alkali-earth particles. Under this conception the size of alkali-earth particles and the thoroughness of their dissemination throughout the soil become an important consideration, for even with uniform distribution, the particles of an economic addition are very sparsely spaced in the soil mass. Based on surface exposure, equivalence in immediate reactive values would be approached by use of larger applications of coarser particles and smaller applications of finer material. But, with a constant carbonate equivalence, on an economic basis, a comparison between hydrated lime and fine and coarse limestone separates would be expected to show a superiority for the finer and more widely dispersed materials during a given time.

This contribution is based upon such a comparison. The amounts of sulfates and nitrates leached through an uncropped soil were used as measures of efficiency in promoting biological activities. Variations caused by incorporation of additions in the surface and subsurface zones were also included in the study.

## EXPERIMENTAL

The soil used was a brown loam of pH 6.38 in a 1-5 aqueous suspension without preliminary extraction (5). The soil was exposed to natural rain-

<sup>1</sup> From data obtained by means of a research fellowship maintained by the National Lime Association and lysimeter equipment donated by the American Limestone Company of Knoxville.

<sup>2</sup> The results of the first 2 years and those of the third and fourth annual periods were obtained, respectively, by Mr. Hanvey Stanford, Mr. T. D. Hardin, and Dr. R. M. Barnette, all formerly fellowship assistants.

fall for 4 years—May, 1921 to May, 1925. Washed separates of high-calcic and dolomitic limestones were used in comparison with a water-slaked high calcic lime. A constant equivalence of 2,000 pounds of  $\text{CaO}$ , or 3,570 pounds of  $\text{CaCO}_3$ , per 2,000,000 pounds of soil, moisture-free basis, was used; but the intensity of treatment per zone was actually twice this amount, since the incorporations were made only with either the upper or lower half of the soil stratum. The total nitrogen and sulfur contents of the soil were originally 0.105 per cent and 0.057 per cent, respectively, moisture-free basis. The nitrogen content of the rainfall was not sufficient to be taken into account; but 180.4 pounds of sulfur was brought down during the 4-year period. The details of treatment, of description and illustration of the equipment, and of outgo of calcium and magnesium during the 4-year period have been given in previous contributions (1, 2).

#### DISCUSSION

##### *Sulfate Outgo*

*Surface-zone incorporations.* The sulfate leachings for the annual and 4-year periods are given in table 1, in which the results are expressed as pounds of S per 2,000,000 pounds of soil. The maximum outgo for the first year was obtained from the hydrated lime. When expressed as  $\text{CaSO}_4$ , the maximum increase in outgo of the first year amounts to 65 pounds, whereas the corresponding maximum for the 4-year period amounts to 88 pounds, or 48.8 per cent of the rainfall increment for the full period. Appreciable total accelerations were induced by the three finer limestone separates and composite, the 40–80-mesh and the 80–200-mesh separates and composite of dolomite. Marked increases were noted more generally during the first year. The 10–20-mesh separates of both limestone and dolomite failed to show any accelerative effect during the first 3 years. As a whole, it might be said that the results from controls and all treatments were quite comparable after the first year.

The total precipitations for the first and second years were 52.52 inches and 52.03 inches, respectively. But with such agreement in rainfall there was a marked decrease in the sulfate outgo both for controls and for treated soils during the second year and a return to a uniformity after that period. This undoubtedly registers the initial abnormality resulting from the thorough mixing and aëration of the soil at the time of placement; but it does not appear whether this was due to actual variation in sulfate production, or to alteration in the retentive capacity of the soil for sulfates.

The beneficial effects of the surface zone incorporations were restricted, in a measure, by the frequent condition of excessive dryness in the upper surface. Furthermore, the generated and leached neutral calcium and magnesium salts may have been partly absorbed during their movement through the untreated subsurface zone, for it was found (1) that the total amount of calcium

TABLE 1  
*Sulfide sulfur leached during 4-year period from surface-sone additions of 2000 pounds of CaO and equivalent limestone and dolomite separates*  
 Terms of S per 2,000,000 pounds moisture-free loam soil

TREATMENT	FIRST ANNUAL PERIOD					SECOND ANNUAL PERIOD					THIRD ANNUAL PERIOD					FOURTH ANNUAL PERIOD				TOTAL FOR 4 YEARS	4-YEAR INCREASE IN OUTPUT OVER CONTROLS
	May to Sep-tember	January to March	March to May	Total	lbs.	May to December	December to February	February to May	Total	May to September	September to February	February to May	Total	May to December	December to May	Total					
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.					
Controls.....	9.5	16.7	17.6	8.1	51.9	11.4	11.8	9.3	32.5	4.8	12.0	8.5	25.3	9.4	16.0	25.4	135.1	.....	+20.7		
Ca(OH) <sub>2</sub> .....	15.4	29.2	14.7	7.9	67.2	13.8	9.3	8.2	31.3	4.1	16.9	6.1	27.1	14.6	15.6	30.2	155.8	+ 6.1	+10.2		
L. S. 10-20.....	6.4	18.1	16.5	8.3	49.3	12.9	10.4	8.8	32.1	3.9	16.0	7.0	26.9	12.3	20.6	32.9	141.2	+15.6	+15.1		
L. S. 20-40.....	6.9	22.1	15.5	7.5	52.0	14.0	11.1	8.9	34.0	4.5	17.5	7.0	29.0	14.8	15.5	30.3	145.3	+15.2	+15.2		
L. S. 40-80.....	7.4	26.4	15.6	7.7	57.1	14.1	10.8	9.0	33.9	4.7	18.2	5.6	28.5	15.3	15.9	31.2	150.7	+14.5	+13.2		
L. S. 80-200.....	10.1	24.1	17.1	9.2	60.5	14.8	10.2	7.6	32.6	5.4	17.1	6.0	28.5	13.3	15.3	28.6	150.2	+14.9	+14.9		
L. S. Comp.....	6.7	27.9	16.3	8.7	59.6	13.7	8.8	8.6	31.1	4.6	17.6	6.6	28.8	14.2	16.6	30.8	150.3	- 1.3	+ 5.2		
Dol. 10-20.....	6.5	17.5	16.2	6.5	46.7	11.5	9.3	9.9	30.7	3.1	15.6	6.6	25.3	11.0	20.1	31.1	133.8	+14.5	+13.2		
Dol. 20-40.....	8.0	20.3	15.3	9.0	52.6	11.9	9.8	8.5	30.2	3.3	16.5	6.2	26.0	13.6	17.9	31.5	140.3	+14.9	+14.9		
Dol. 40-80.....	7.1	23.4	16.6	8.5	55.6	12.3	11.4	10.5	34.2	4.1	17.4	6.7	28.2	13.8	17.8	31.6	149.6	+13.2	+14.9		
Dol. 80-200.....	7.8	26.6	15.3	9.3	59.0	12.1	10.6	8.4	31.1	4.0	16.3	7.4	27.7	13.9	16.6	30.5	148.3	+14.9	+14.9		
Dol. Comp.....	7.7	24.0	14.9	7.3	53.9	13.8	11.3	9.9	35.0	4.0	17.4	7.2	28.6	14.9	17.6	32.5	150.0	+14.9	+14.9		



TABLE 2  
*Sulfate sulfur leached during 4-year period from subsurface-zone additions of 2,000 pounds of CaO and equivalent limestone and dolomite separates*  
 Terms of S per 2,000,000 pounds moisture-free loam soil

TREATMENT	FIRST ANNUAL PERIOD						SECOND ANNUAL PERIOD				THIRD ANNUAL PERIOD				FOURTH ANNUAL PERIOD				TOTAL FOR 4 YEARS	4-YEAR INCREASE IN OUTGO OVER CONTROLS
	May to September	September to January	January to March	March to May	Total		May to December	December to February	February to May	Total	May to September	September to February	February to May	Total	May to December	December to May	Total			
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.		
Controls.....	9.5	16.7	17.6	8.1	51.9	11.4	11.8	9.3	32.5	4.8	11.9	8.5	25.2	9.4	16.0	25.4	135.1	....		
Ca(OH) <sub>2</sub> .....	28.9	22.8	9.6	5.9	67.2	13.2	7.8	6.1	27.1	5.7	12.7	6.7	25.1	12.6	16.5	29.1	148.5	+13.4		
L. S. 10-20.....	9.8	19.9	14.6	10.3	54.6	13.7	9.3	9.4	32.4	6.7	14.2	7.2	28.1	13.0	13.6	26.6	141.7	+6.6		
L. S. 20-40.....	12.4	26.1	13.3	10.4	62.2	16.4	10.3	8.6	35.3	5.9	15.3	7.5	28.7	14.4	17.8	32.2	158.4	+23.3		
L. S. 40-80.....	19.5	25.0	11.2	6.0	61.7	15.0	8.8	6.8	30.6	5.9	13.2	7.4	26.5	12.8	20.1	32.9	151.7	+16.6		
L. S. 80-200.....	23.1	24.8	9.7	6.1	63.7	12.6	7.4	7.4	27.4	4.8	12.1	7.0	23.9	11.5	14.9	26.4	141.4	+6.3		
L. S. Comp.....	16.7	25.2	11.9	7.6	61.4	14.9	8.6	7.9	31.4	6.3	13.9	6.3	26.5	12.9	17.8	30.7	150.0	+14.9		
Dol. 10-20.....	8.3	18.5	15.4	7.7	49.9	16.4	8.4	9.0	33.8	4.2	14.3	6.9	25.4	10.6	17.2	27.8	136.9	+1.8		
Dol. 20-40.....	8.2	23.9	13.3	7.4	52.8	15.7	6.9	7.9	30.5	4.1	14.5	7.4	26.0	12.7	16.3	29.0	138.3	+3.2		
Dol. 40-80.....	11.8	27.4	12.4	7.5	59.1	15.7	9.7	8.2	33.6	6.6	14.6	10.5	31.7	12.9	17.1	30.0	154.4	+19.3		
Dol. 80-200.....	16.3	26.3	11.6	6.3	60.5	15.0	8.2	6.2	29.4	6.6	12.6	7.1	26.3	12.0	15.7	27.7	143.9	+8.8		
Dol. Comp.....	12.8	25.6	11.8	6.8	56.9	15.0	10.4	8.7	34.1	5.4	15.8	8.6	29.8	13.8	19.2	33.0	153.8	+18.7		

and magnesium which passed out from the surface zone was materially reduced in that way. To offset this absorption exerted by the lower zone, however, some beneficial effect from bicarbonates carried down to the untreated subsurface zone from the surface zone, may be expected.

Although the sulfur outgo from every treatment, save 10-20-mesh dolomite, was greater than that from the untreated soil, total outgo was in no case equal to the sulfate sulfur brought down by rain waters.

*Subsurface-zone incorporations.* The tanks which received subsurface-zone additions (table 2) may be regarded as having yielded a constant amount of sulfates from the untreated surface zone. The leaching variations induced by additions to the lower zone may therefore be considered as due solely to activities within that zone.

Again, as in the case of the surface-zone additions, the hydrated lime produced the largest outgo during the first year, but not thereafter. During the first year, 10 of the 11 additions yielded sulfates in excess of the outgo from the controls. During the 4-year period 3 of the limestone additions and 2 of the dolomite separates caused sulfate losses in excess of the outgo from the hydrated lime. Previous results (2) demonstrated the fact that all limestone separates were much more extensively, and presumably more rapidly, disintegrated in the lower zone. As this tended to minimize the influence of variations in size of particles in the lower zone, no consistent differences appear. The lime treatment, two of the limestone, and two of the dolomite additions gave losses less than those from corresponding surface-zone incorporations, although the reverse was true for 3 of the limestone and 3 of the dolomite additions; but in 4 cases the differences were small.

As in the case of the surface-zone additions all treatments, save 10-20-mesh dolomite, gave appreciable accelerations in sulfate outgo; but, again in no case did the total for 4 years equal the rainfall supply of sulfates.

### *Nitrate Outgo*

*Surface-zone incorporations.* With the exception of the 10-20-mesh limestone separate, all additions resulted in nitrate leachings in excess of the outgo from the control for the first year (table 3). During the same period the two finer limestone separates and the hydrated lime yielded the largest increases, and the finer limestone separates were more accelerative than were the corresponding dolomite separates. In the second year each limestone separate was more accelerative than its corresponding dolomite separate in promoting nitrate outgo. During this period of each of four limestone separates, three of the dolomite separates and their composite gave losses greater than the outgo from hydrated lime. During the third year the hydrated lime yielded less of nitrates than did the control, but increased outgo was obtained from all separates and both composites. With the exception of the dolomite composite the same was true also for the fourth year.

TABLE 3  
*Nitrate N leached during 4-year period from surface-zone additions of 2,000 pounds of CaO and equivalent limestone and dolomite separates*  
 Terms of N per 2,000,000 pounds of moisture-free loam soil.

TREATMENT	FIRST ANNUAL PERIOD					SECOND ANNUAL PERIOD				THIRD ANNUAL PERIOD				FOURTH ANNUAL PERIOD			TOTAL FOR 4 YEARS	4-YEAR INCREASE IN OUTGO OVER CONTROLS
	May to September	September to January	January to March	March to May	Total	May to December	December to February	February to May	Total	May to September	September to February	February to May	Total	May to December	December to May	Total		
	lbs.	sq.	sq.	sq.	lbs.	sq.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
Controls.....	20.8	32.1	3.3	6.9	63.1	32.4	2.3	2.4	37.1	21.1	11.2	1.8	34.1	29.6	5.1	34.7	169.0	....
Ca(OH) <sub>2</sub> .....	28.9	47.6	4.9	6.7	88.1	41.7	3.5	1.6	46.8	20.9	4.1	3.3	28.3	29.6	2.3	31.9	195.1	+26.1
L. S. 10-20.....	22.4	30.4	4.3	4.9	62.0	41.2	1.3	1.6	44.1	21.9	11.8	3.2	36.9	33.6	4.4	38.0	181.0	+12.0
L. S. 20-40.....	25.9	41.9	3.7	6.1	77.6	51.4	4.3	2.9	58.6	28.8	13.5	3.3	45.6	38.5	6.3	44.8	226.6	+57.6
L. S. 40-80.....	36.7	44.6	3.9	7.1	92.3	59.9	3.4	3.6	66.9	28.1	11.6	2.8	42.5	37.4	5.0	42.4	244.1	+75.1
L. S. 80-200.....	34.1	53.2	2.7	4.2	94.2	54.2	3.2	3.4	60.8	32.0	12.3	3.3	47.6	30.7	5.0	35.7	238.3	+69.3
L. S. Comp.....	26.6	37.9	4.6	5.8	74.9	22.5	2.5	1.0	26.0	24.7	10.3	3.2	38.2	32.6	2.7	35.3	174.4	+ 5.4
Dol. 10-20.....	23.9	38.8	2.6	3.2	68.5	30.9	3.6	2.4	36.9	22.6	19.6	3.4	45.6	36.1	5.3	41.4	192.4	+23.4
Dol. 20-40.....	26.4	39.2	2.8	5.3	73.7	43.3	3.5	2.5	49.3	27.2	14.9	3.4	45.5	36.6	6.0	42.6	211.1	+42.1
Dol. 40-80.....	21.7	41.9	3.4	3.9	70.9	47.0	2.9	1.9	51.8	25.9	16.8	3.5	46.2	33.6	5.7	39.3	208.2	+39.2
Dol. 80-200.....	29.1	44.7	3.4	2.8	80.0	49.7	2.9	2.7	55.3	32.9	17.9	4.0	54.8	31.3	5.8	37.1	227.2	+58.2
Dol. Comp.....	24.7	41.0	3.4	4.5	73.6	48.4	3.9	2.6	54.9	27.4	15.9	3.3	46.6	29.4	4.8	34.2	209.3	+40.3

TABLE 4  
*Nitrate N leached during 4-year period from subsurface-zone additions of 2,000 pounds of CaO and equivalent limestone and dolomite separates*  
 Terms of N per 2,000,000 pounds of moisture-free loam soil

TREATMENT	FIRST ANNUAL PERIOD					SECOND ANNUAL PERIOD					THIRD ANNUAL PERIOD					FOURTH ANNUAL PERIOD				TOTAL FOR 4 YEARS	4-YEAR INCREASE IN OUTGO OVER CONTROLS
	May to Sep	September to January	January to March	March to May	Total	May to Decem	December to February	February to May	Total	May to September	September to February	February to May	Total	May to December	December to May	Total	May to December	December to May	Total		
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.		
Controls.....	20.8	32.1	3.3	6.9	63.1	32.4	2.3	2.4	37.1	21.1	11.2	1.8	34.1	29.6	5.1	34.7	169.0	.....	lbs.	.....	lbs.
Ca(OH) <sub>2</sub> .....	38.9	35.7	3.6	6.4	84.6	26.7	1.4	0.8	28.9	24.9	3.1	3.2	31.2	26.6	2.3	28.9	173.6	+ 4.6	.....	+ 4.6	lbs.
L. S. 10-20.....	19.4	35.8	5.4	7.4	68.0	47.0	3.1	1.2	51.3	22.7	12.2	3.1	38.0	33.8	5.0	38.8	196.1	+27.1	.....	+27.1	lbs.
L. S. 20-40.....	36.9	43.8	3.4	5.3	89.4	50.0	3.9	3.1	57.0	25.0	10.6	2.9	38.5	35.1	4.9	40.0	224.9	+55.9	.....	+55.9	lbs.
L. S. 40-80.....	30.8	48.8	4.6	6.2	90.4	49.3	4.1	2.7	56.1	26.6	8.3	3.6	38.5	29.3	5.0	34.3	219.3	+50.3	.....	+50.3	lbs.
L. S. 80-200.....	30.0	48.7	3.5	3.5	85.7	35.3	2.5	1.5	39.3	24.4	5.8	3.4	33.6	30.4	5.2	35.6	194.2	+25.2	.....	+25.2	lbs.
L. S. Comp.....	31.0	31.0	4.0	4.8	70.8	35.7	1.4	1.1	38.2	21.5	5.8	3.1	30.4	26.5	3.4	29.9	169.3	+ 0.3	.....	+ 0.3	lbs.
Dol. 10-20.....	32.6	36.2	2.6	5.0	76.4	41.1	3.3	1.7	46.1	29.4	13.5	3.6	46.5	24.7	5.2	29.9	198.9	+29.9	.....	+29.9	lbs.
Dol. 20-40.....	26.3	36.5	3.3	5.3	71.4	50.3	2.7	2.1	55.1	23.4	17.9	4.0	45.3	32.4	5.0	37.4	209.2	+40.2	.....	+40.2	lbs.
Dol. 40-80.....	34.2	37.9	3.1	5.6	80.8	39.2	5.5	3.4	48.1	32.2	14.9	5.2	52.3	25.9	5.1	31.0	212.2	+43.2	.....	+43.2	lbs.
Dol. 80-200.....	32.8	46.3	2.9	5.3	87.3	50.0	2.2	2.8	55.0	37.0	14.9	4.1	56.0	29.2	5.3	34.5	232.8	+63.8	.....	+63.8	lbs.
Dol. Comp.....	29.6	40.9	2.4	6.1	79.0	48.6	4.3	2.5	54.9	29.4	17.0	3.4	49.8	32.2	6.0	38.2	221.9	+52.9	.....	+52.9	lbs.

For the 4-year period the 20-40-, 40-80-, and 80-200-mesh separates of both limestone and dolomite gave nitrate losses in excess of the outgo from the hydrated lime treatment. The differences between the influence of 10-20-mesh separate and that of the finer separates are quite marked for both limestone and dolomite. The finer separates of limestone proved more accelerative than the corresponding dolomite separates; but for some reason unexplained, the reverse was true of the composites. As was the case with the sulfates, there was a distinct decrease after the first year; but, in general, the enhanced outgo was continuous throughout the 4-year period.

With the exception of the first year, the losses from May to December constituted the larger fractions of the several annual losses. In this respect nitrification differed from sulfonation, since the generation of sulfates was evenly distributed throughout the year.

*Subsurface-zone incorporations.* The hydrated lime addition gave an enhanced outgo, approximately the same as that from the surface incorporation during the first annual period, but the reverse was true during the succeeding three years, so that, differing from the same addition to the surface zone, the total outgo was only slightly greater than that from the controls (table 4).

The correlation between nitrification and disintegration of separates may be seen more readily by reference to the determinations of residual carbonates, as given in a preceding contribution (2). All of the limestone and dolomite additions caused increased nitrate outgo during the first year, but the influence of fineness was not so apparent as in the upper-zone additions. In general the same may be said of the results for the third and fourth years. In the totals there was found the same superiority of dolomite composite over the limestone composite noted in the surface-zone incorporations. Peculiarly, the relation between increasing outgo and progressive fineness was more definitely shown in the case of the more slowly disintegrated dolomite than in the case of the more rapidly disintegrated limestone. The 80-200-mesh limestone fell behind the 40-80-mesh separate during the first 3 years and the two were practically equivalent for the fourth year, so that the 4-year outgo from the finer separate was decidedly less.

In agreement with the sulfate outgo, the 4-year nitrate yield from the hydrated lime addition was much less than that from the same incorporation in the surface zone. The lower-zone addition could hardly affect the overlying untreated zone. The same addition in the upper zone, however, exerted its effect not only there, but also in the lower zone because of the bicarbonates leached to it. The three finer high-calcic limestone separates had the same effect, but the reverse was true of the 10-20-mesh separate which had been more extensively disintegrated in the lower zone (2). Neither surface-zone, nor subsurface-zone incorporations of the limestone composite evidenced appreciable effect upon nitrate outgo; but both incorporations of dolomite exerted a distinct effect, the lower-zone addition having been the

more active. No large or consistent differences appeared to be attributable to the several dolomite separates as influenced by zone of incorporation.

#### SUMMARY

The maximum enhancements in average annual leachings of sulfur and nitrogen from the uncropped soil were only 5.8 pounds and 18.8 pounds, respectively. Those amounts would probably have been still further reduced had non-legumes been grown during the 4-year period. Judged by its pH value, the soil is not to be considered as strongly acid, but it responds to moderate liming in the field and it has shown capacity to disintegrate twice as much  $\text{CaCO}_3$  (2) as that supplied by the 3570-lb. equivalence from  $\text{CaO}$ , and finely divided limestone and dolomite. In the light of those characteristics, certain of the sulfate and nitrate findings are consistent while others are not, in spite of the definite correlation between the disintegrations (2) and the calcium-magnesium outgo (1) as influenced by form, fineness and zone-of-incorporation. In a sense, the sulfate results may be considered as showing the influence of the additions upon the soil's tendency to retain sulfates, since in no case was the sulfate outgo equivalent to the rainfall increment of sulfate sulfur.

In the surface incorporation group  $\text{Ca}(\text{OH})_2$  was most accelerative upon sulfate outgo. The finer separates of limestone and dolomite were comparable and both were more potent than the corresponding coarser materials, the greatest differences having occurred during the first year. In nitrate production the coarser separates had less effect than the finer separates and those in turn caused nitrate leachings in excess of those from the hydrated lime. Each of the finer limestone separates was more active than its corresponding dolomite separate, but the reverse was true of the 10-20-mesh and composite additions.

In the subsurface addition group the influence of form and availability, governed by fineness, was not so consistent in producing acceleration of sulfate outgo. In nitrate losses, 9 of the 10 limestone and dolomite separates gave leachings in excess of the outgo from  $\text{Ca}(\text{OH})_2$ . In this zone of greater carbonate disintegration, the influence of increase in surface of separates was not so marked, nor was the relationship between limestone and dolomite so well established.

As a whole, excluding the 10-20-mesh separates, it appears that neither degree of fineness of 2000-pound  $\text{CaO}$  equivalences of  $\text{Ca}(\text{OH})_2$ , of limestone, and of dolomite, nor depth of incorporation was uniformly consistent in its influence upon the total losses of sulfates and nitrates from this particular soil during the 4-year period, under the prevailing climatic conditions.

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## SOME SOIL AND PLANT RELATIONSHIPS

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It has been stated previously that water is one of the best indices of several soil characteristics, such as texture, structure, colloidal content, and activation of surfaces (1). A study of the moisture relationships of plants grown under different conditions appears to be profitable. Results reported by McCool and Millar (2) from the use of the dilatometer method raised the question as to the effect of the composition of soil solution upon the amount of easily freezable water in plant tissue. Later the question was raised with respect to the use of the heat of wetting method in studying the moisture relationships of soils and plants. Accordingly samples of alfalfa were taken at different periods during the growing seasons of 1924 and 1925 from certain plots of one of our fertility fields. In addition samples of other crops growing on differently treated muck and mineral soils were collected.

### THE EFFECT OF DIFFERENT TREATMENTS ON HEAT OF WETTING

Samples of alfalfa were exposed 24 hours to four different temperatures and the calories per gram liberated upon wetting were determined. The results obtained are presented in table 1.

The data show that some heat is evolved when this plant tissue is brought into contact with water after it has been exposed to a temperature of 22°C.; about twice this amount, after it has been held at a temperature of 60°C.; four times as much, in case of the 90°C. exposure; and a relatively slight increase in amount, with the further 10°C. rise of temperature.

Samples of alfalfa, soybeans and clover were leached until the freezing point depressions, as determined by the Beckmann thermometer, were very low. The samples were then dried in the oven at 96°C. The heat of wetting determinations were made upon these samples and also upon samples that had not been leached. According to the data in table 2 the heat of wetting of these plant materials increases slightly upon leaching. It appears that the heat of wetting is due in the main to the insoluble substances—probably colloidal in nature—in the tissue. When leached, the proportion of these in a given amount of material increases. This condition probably brings about increases in the heat of wetting.



## HEAT OF WETTING OF DIFFERENT MATERIALS

Before taking up the effects of different kinds of soils and their treatment on the water relationships, the heat of wetting of a number of plants, leaves, roots, and seeds, and commercial corn starch was determined. The calories of heat given off per gram of each of the materials employed are given in table 3.

It is to be noted that the samples of corn starch yielded the highest heat of wetting, and that of the seeds, the wheat, rye, and corn are next in order, with Canadian field pea, field bean, and alfalfa slightly lower. Other materials that yielded similar results were soybean, sweet clover, white clover, and red clover. Of the remaining materials studied, the leaves of peach and apple trees give strikingly low results.

TABLE 1  
*The effect of temperature on heat of wetting of alfalfa*

TEMPERATURE	HEAT OF WETTING
°C.	<i>calories per gram</i>
22	3.85
60	7.85
90	15.03
100	16.05

TABLE 2  
*The effect of leaching upon the heat of wetting*

KIND OF PLANT	UNLEACHED	LEACHED
	<i>calories</i>	<i>calories</i>
Alfalfa.....	15.31	17.97
Red clover.....	15.80	17.62
Soybeans.....	17.70	19.81

## THE EFFECT OF FERTILIZATION ON WATER RELATIONSHIPS OF PLANTS

Since the indications are that the nature of the plant materials affects the heat of wetting somewhat, it was considered advisable to determine whether the fertilization of soils affects the water relationships of the crops grown on them. On August 10, samples of leaves were taken from sugar beets, chicory, and white clover growing on differently fertilized muck plots; and on July 28, from soybeans growing on mineral soil. Samples of parsnip roots, turnip roots, table beet roots, and celery leaves were collected on November 5. The essential data are given in table 4.

The results show that the leaves of the plants grown on the unfertilized muck soil—notably sugar beet, chicory, celery, and white clover—have a higher heat of wetting than those taken from the differently fertilized plots; whereas the soybean leaves of plants grown on mineral soil give similar but somewhat

less striking results. Negative results were obtained from alfalfa and sweet clover tops and also from beet, parsnip, and turnip roots.

Although fertilization did not affect the amount of heat evolved by alfalfa when moistened, it is apparent that the time of cutting and consequently the

TABLE 3  
*The heat of wetting of different materials*

MATERIAL	HEAT OF WETTING
	<i>calories per gram</i>
Cornstarch.....	20.1
Wheat.....	18.8
Rye.....	18.6
Corn grain.....	17.8
Field pea.....	17.3
Field bean.....	15.6
Alfalfa seeds.....	15.1
Soybean leaves.....	17.9
Sweet clover tops.....	16.1
White clover tops.....	15.8
Red clover leaves.....	16.0
Alfalfa roots.....	14.0
Turnip roots.....	9.2
Maple leaves.....	15.3
Beech leaves.....	13.8
Oak leaves.....	13.7
Cherry leaves.....	13.6
Mature Kentucky Bluegrass.....	15.2
Young Kentucky Bluegrass.....	13.4
Apple leaves.....	10.1
Peach leaves.....	8.9
Celery leaves.....	11.1
Parsnip roots.....	9.7

TABLE 4  
*The effect of fertilisation on the heat of wetting*

MATERIAL	TREATMENT				
	O	K	P	PK	NPK
Sugar beet leaves.....	9.7	5.5	7.3	3.8	....
Chicory leaves.....	16.9	15.8	18.4	12.5	....
Celery leaves.....	11.4		10.6	10.4	9.2
Soybean leaves.....	17.1		15.8	16.8	
White clover.....	17.2			15.9	13.7

composition of the plant does affect it. Grimm alfalfa sampled on May 21, 1924 gave a heat of wetting of 14.2 calories per gram, whereas on June 26 it was 17.7 calories. Similar results were obtained with Cossack and the common variety of alfalfa and also with Kentucky bluegrass.

It would be interesting to determine whether there is any relationship between soil fertilization and heat of wetting and the loss of water from plants when harvested. It is probable that the concentration of the cell sap, as affected by the treatment afforded the soil, plays an important rôle in such losses, perhaps more so than the colloids present as indicated by the heat of wetting method. M. F. Mason<sup>1</sup> determined the amount of easily freezable water in moistened leaves of white clover and soybean grown on fertilized and unfertilized soil and found it to be greater in those taken from the unfertilized areas. On the other hand when these were leached with distilled water the results obtained were reversed.

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<sup>1</sup> Unpublished data, this laboratory.

## SOME EFFECTS OF MULCHING PAPER ON HAWAIIAN SOILS

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### INTRODUCTION

The use of mulching paper upon Hawaiian crops was first started by C. F. Eckart, Manager of Olaa Sugar Company, at Olaa, Hawaii, in the year 1914. The lands of the Olaa Sugar Company are located largely in the windward section of the Island of Hawaii, in a region of high annual rainfall. The average yearly precipitation ranges from 150 to 200 inches on most of the plantation fields. This rainfall tends to cause a heavy growth of weeds, so that weed control was originally one of the heaviest expenses of this plantation.

Eckart first applied mulching paper for weed control to the "kua-kua" or portion of the cane furrow which lies between the actual rows of cane. This middle part of the cane furrow is ordinarily kept clear from weeds by animal or hand cultivation until the cane is large enough to shade the ground and prevent weed development. This use of mulching paper proved effective in reducing weed growth in the middle portion of the furrow, but left the weeds a chance to develop around the cane stalks. Here weeds could only be removed by hand hoeing.

In 1916 the process was modified by Eckart, and "row mulching" was introduced. In this method of using the paper mulch, a light weight tar or asphalt impregnated paper was spread directly over the row of seed cane or harvested stubble. When the young cane shoots first appeared they were extremely sharp and those that grew upright readily pierced the mulching paper. Some of the shoots that grew diagonally, however, would commence to unfold under the paper and after four to six weeks it was necessary to slit the mulching paper. By this time practically all the weeds under the paper had been killed, whereas the cane shoots were unharmed.

The mulching paper used at Olaa was 3 feet wide. This left an uncovered space in the center of the cane row. The cane trash was collected into this space before the paper was laid and served as an effective mulch to keep down most of the weeds. As the labor required in weed control is greatly reduced by the use of the paper mulch, it is now the standard plantation practice at Olaa Sugar Company. An ideal paper for use on sugar cane is manufactured at Olaa from bagasse and wood pulp.

It was observed in the early work at Olaa that the paper mulch appeared to exert some other effect upon the crop, in addition to preventing injury from weed growth. Paper mulched plots made a noticeably better growth than adjoining plots which were kept equally free from weeds. This difference was especially noticeable during the early period of growth before the cane had "closed in" and completely shaded the soil. Eckart attributed this to added soil warmth and to moisture conservation due to the paper. Larsen (2) reported that the soil under the paper mulch was from 3 to 6° higher in temperature than bare soil.

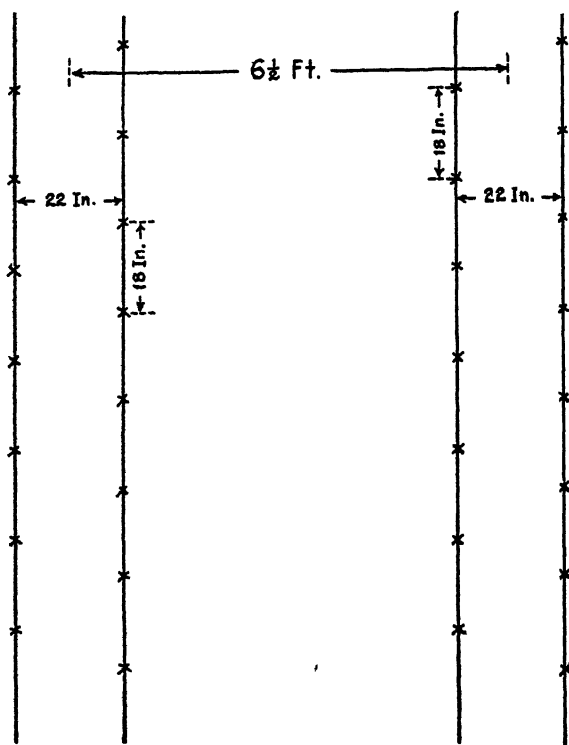


FIG. 1. DOUBLE-ROW SCHEME FOR PLANTING PINEAPPLES

The favorable effect upon the growth of sugar cane suggested the trial of the paper mulch upon the pineapple crop. Trial plantings were made by the Hawaiian Pineapple Company in 1919 and 1920. The growth obtained under the paper mulch was notably better than that made by adjacent unmulched plots. As a result of these experiments the Hawaiian Pineapple Company in 1922 purchased Echart's patent rights for the use of mulching paper upon the pineapple crop in Hawaii. At the present time the great majority of the pineapples grown in Hawaii are planted in mulching paper.

Because of the widespread use of mulching paper with pineapples, it appeared desirable to obtain more detailed information as to the effect of the paper mulch upon the soil. The experiments reported here were carried out as part of the chemical work performed by this Experiment Station for the Association of Hawaiian Pineapple Canners during the years 1921 to 1924 inclusive.

In the Hawaiian Islands pineapples are ordinarily planted in a 2-row system, which is indicated in figure 1. The distance between the pairs of rows is usually  $6\frac{1}{2}$  feet from center to center. The individual rows of each pair are commonly 22 inches apart and the plants are set in a staggered effect 18 inches apart in the row. The usual procedure is to apply a mixed fertilizer at the rate of 500 to 750 pounds per acre on the line of the row before laying the paper. The method of laying the paper is illustrated in plate 1, figure 1. The plants are then inserted through the paper so that a new planted field will appear as in plate 1, figure 2.

With the pineapple crop it is considered desirable to have the mulching paper last as long as possible. The paper used is therefore a tar or asphalt impregnated and coated paper similar to a light or medium grade of building paper. This type of material will ordinarily last until one or more ratoon crops have been harvested.<sup>1</sup> Before the introduction of the paper mulch it was unusual to obtain more than two ratoon crops, but it is now hoped that the larger and more vigorous plants grown under the paper mulch may ratoon for a larger period of time.

#### EXPERIMENTAL

The studies carried out in this investigation have been concerned with determining the effect of the paper mulch upon soil temperature, soil moisture, and ammonification and nitrification of the soil. All of these observations were made under field conditions.

#### *Effect of paper mulch upon soil temperature*

The work on soil temperature has extended over a period of more than two years and has consisted of determining the temperature of the bare soil and of comparison with paper mulched plots. During the first part of the work, no pineapple plants were grown, as it was desired to find the effect of the mulching paper on the soil with the varying weather conditions that occur at different seasons of the year. After temperatures in mulched and unmulched soil were followed throughout an entire season, pineapple plants were put in and the observations were continued.

The temperatures were recorded by combined soil and air thermographs.

<sup>1</sup> It may be explained that on the pineapple plantations in Hawaii it is customary to speak of the first crop of pineapples produced in about two years after planting as the "plant crop." The successive crops of fruit produced each year after the plants come in bearing are known as the first, second or third ratoons.

Both Julien P. Friez and J. Richard instruments were used in the work. The soil elements were buried to a depth of 4 inches below the surface. The reading of the soil graphs was checked by placing accurately standardized glass thermometers alongside the soil elements, and reading these thermometers at short intervals until the thermographs and thermometers were in close agreement. After the instruments were in good adjustment, it was found sufficient to check the soil and air elements against accurate thermometers at periods of one or two days.<sup>2</sup>

The data obtained in these temperature studies were so voluminous that it appeared to be feasible to select only certain typical weekly periods to exemplify the changes in soil temperature caused by the paper mulch. Table 1 gives such a weekly record obtained at the Makiki plot of this Experiment Station located in Honolulu. The period was March 5 to 12, 1923, before the recording instruments were transferred to Wahiawa. In this period of the spring, the weather was very variable. It was found immediately that the effect of the paper mulch varied with the weather conditions. During clear, bright weather at this time of the year, the temperature of the soil under paper was generally about 4 to 6°F. warmer, during the middle of the day and afternoon, than the unmulched soil. The temperature of the mulched soil continued to be higher than that of the unmulched plots, even in the night and early morning, though the difference decreased during this night period.

The influence of rainfall was noted early, for rain fell on several days during the period covered in table 1. The rain decreased the difference between the bare soil and the paper mulched plots. In fact it was found later that for a short time after heavy rains the bare soil might be warmer than the soil under the paper mulch. Further work at the Makiki station dealt with the effect of shade. A lath screen was erected over the mulched and unmulched plots and it was found that the effect of the paper mulch on the soil temperature varied directly with the exposure to the sun's rays. When the soil was either half or more shaded, the effect of the paper mulch upon the soil temperature was greatly reduced.

The work was then transferred to Wahiawa in a typical pineapple district where the experimental plots were located at the Experiment Station of the Hawaiian Pineapple Cannery Association. The work upon bare plots with and without paper was continued in order to discover the effect of the paper mulch at different seasons of the year.

During the early summer, the differences were of somewhat the same order as those previously observed in Honolulu. A typical period during late May is given in table 2. As the season progressed and the maximum air tempera-

<sup>2</sup> The major portion of the soil temperature records was made at the Experiment Station of the Association of Hawaiian Pineapple Cannery, located at Wahiawa, Oahu. The collection of the records was made by the agricultural department of that station. The photographs illustrating this article were furnished us by H. L. Denison, Agriculturist of the Pineapple station.

TABLE 1

*Soil temperatures in ordinary cane furrows*

Makiki Station, Hawaiian Sugar Planters' Association, March 5 to 12, 1923

DATE	HOUR	WEATHER	TEMPERATURE OF AIR	TEMPERATURE OF SOIL		DIFFERENCE DUE TO PAPER
				No paper	Under paper	
March 5	12 noon	Fair	°F. 80	°F. 77	80	3
	2 p.m.		80	81	87	6
	4 p.m.		78	81	87	6
	6 p.m.		76	81	86	5
	8 p.m.		74	79	83	4
	10 p.m.		73	77	81	4
	12 midnight		73	75	80	5
March 6	2 a.m.	Cloudy during part of day	73	73	78	5
	4 a.m.		71	73	77	4
	6 a.m.		72	71	76	5
	8 a.m.		73	71	75	4
	10 a.m.		75	72	76	4
	12 noon		76	74	79	5
	2 p.m.		77	78	83	5
	4 p.m.		76	81	85	4
	6 p.m.		73	81	83	2
	8 p.m.		72	77	81	4
	10 p.m.		71	75	79	4
	12 midnight		70	73	78	5
March 7	2 a.m.	Cloudy during part of day	70	73	76	3
	4 a.m.		71	73	75	2
	6 a.m.		71	71	75	4
	8 a.m.		72	71	74	3
	10 a.m.		75	71	75	4
	12 noon		76	73	76	3
	2 p.m.		76	75	79	4
	4 p.m.		73	77	79	2
	6 p.m.		71	75	78	3
	8 p.m.		68	73	76	3
	10 p.m.		68	72	74	2
	12 midnight		68	71	73	2
March 8	2 a.m.	Cloudy and showers	69	71	73	2
	4 a.m.		69	71	72	1
	6 a.m.		69	70	69	-1
	8 a.m.		69	69	69	0
	10 a.m.		71	69	71	2
	12 noon		70	70	78	8
	2 p.m.		75	73	83	10
	4 p.m.		73	75	82	7
	6 p.m.		72	75	80	5
	8 p.m.		70	73	68	-5
	10 p.m.		71	72	69	-3
	12 midnight		71	71	70	-1



TABLE 1—*Continued*

DATE	HOUR	WEATHER	TEMPERATURE OF AIR	TEMPERATURE OF SOIL		DIFFERENCE DUE TO PAPER
				No paper	Under paper	
March 9	2 a.m.	Cloudy and rain	°F. 70	°F. 70	°F. 70	°F. 0
	4 a.m.		70	70	70	0
	6 a.m.		69	69	68	-1
	8 a.m.		71	68	68	0
	10 a.m.		74	69	70	1
	12 noon		71	70	72	2
	2 p.m.		71	71	72	1
	4 p.m.		74	72	73	1
	6 p.m.		71	72	72	0
	8 p.m.		70	71	71	0
	10 p.m.		68	70	71	1
	12 midnight		68	69	70	1
March 10	2 a.m.	Part cloudy	67	69	68	-1
	4 a.m.		69	68	68	0
	6 a.m.		69	67	68	1
	8 a.m.		70	67	68	1
	10 a.m.		73	67	69	2
	12 noon		76	68	72	4
	2 p.m.		76	71	76	5
	4 p.m.		75	74	79	5
	6 p.m.		73	76	79	3
	8 p.m.		69	75	76	1
	10 p.m.		68	73	74	1
	12 midnight		68	72	73	1
March 11	2 a.m.	Fair	67	70	71	1
	4 a.m.		67	70	70	0
	6 a.m.		67	69	70	1
	8 a.m.		67	68	70	2
	10 a.m.		71	68	72	4
	12 noon		78	69	76	7
	2 p.m.		77	72	81	9
	4 p.m.		77	75	83	8
	6 p.m.		73	79	81	2
	8 p.m.		69	79	78	-1
	10 p.m.		67	76	76	0
	12 midnight		66	73	75	2
March 12	2 a.m.	Showers	62	73	73	0
	4 a.m.		60	71	72	1
	6 a.m.		60	70	71	1
	8 a.m.		76	68	70	2
	10 a.m.		78	71	72	1
	12 noon		79	73	76	3
	2 p.m.		78	79	82	3
	4 p.m.		77	79	84	5
	6 p.m.		74	77	83	6
	8 p.m.		68	74	81	7
	10 p.m.		65	72	78	6
	12 midnight		62	70	76	6

TABLE 2

*Soil temperatures at Wahiawa—early summer, May 21 to 28, 1923*

DATE	HOOR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
May 21	2 p.m.	Clear	81	82	88	6
	4 p.m.		78	83	88	5
	6 p.m.		74	81	85	4
	8 p.m.		71	78	82	4
	10 p.m.		70	76	80	4
May 22	12 midnight	Clear	69	74	77	3
	2 a.m.		69	73	75	2
	4 a.m.		68	72	74	2
	6 a.m.		66	71	72	1
	8 a.m.		75	72	73	1
	10 a.m.		80	74	78	4
	12 noon		82	80	88	8
	2 p.m.		83	85	90	5
	4 p.m.		80	85	89	4
	6 p.m.		76	83	87	4
	8 p.m.		72	81	84	3
	10 p.m.		72	78	82	4
	12 midnight		70	76	79	3
	2 a.m.		70	75	77	2
	4 a.m.		69	74	76	2
May 23	6 a.m.	Clear	69	73	75	2
	8 a.m.		75	73	75	2
	10 a.m.		80	74	82	8
	12 noon		82	77	88	11
	2 p.m.		82	83	90	7
	4 p.m.	Clear	78	84	90	6
	6 p.m.		75	83	87	4
	8 p.m.		71	81	83	2
	10 p.m.		70	78	81	3
	12 midnight		70	76	79	3
	2 a.m.	Clear	70	74	77	3
	4 a.m.		69	73	76	3
	6 a.m.		70	73	75	2
	8 a.m.		77	72	75	3
	10 a.m.		80	73	79	6
	12 noon		80	75	83	8
	2 p.m.		78	78	85	7
	4 p.m.		75	79	83	4
	6 p.m.		72	79	81	2
	8 p.m.		71	77	78	1
May 24	10 p.m.		69	75	76	1
	12 midnight	Showers	70	74	74	0
	2 a.m.		71	72	73	1
	4 a.m.		69	71	72	1
	6 a.m.		69	70	71	1
May 25						

TABLE 2—Continued

DATE	HOUR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
May 25	8 a.m.	Showers	72	69	72	3
	10 a.m.		73	70	72	2
	12 noon		74	73	75	2
	2 p.m.		75	75	78	3
	4 p.m.		76	76	80	4
	6 p.m.		72	76	79	3
	8 p.m.		71	74	77	3
	10 p.m.		71	73	75	2
	12 midnight		70	72	73	1
May 26	2 a.m.	Clear	69	71	72	1
	4 a.m.		67	70	71	1
	6 a.m.		70	68	69	1
	8 a.m.		76	68	71	3
	10 a.m.		77	71	76	5
	12 noon		78	73	79	6
	2 p.m.		78	75	82	7
	4 p.m.		75	77	82	5
	6 p.m.		70	76	80	4
	8 p.m.		69	75	77	2
May 27	10 p.m.	Clear	69	74	75	1
	12 midnight		68	72	73	1
	2 a.m.		68	71	72	1
	4 a.m.		67	70	71	1
	6 a.m.		70	69	70	1
	8 a.m.		75	68	72	4
	10 a.m.		77	70	77	7
	12 noon		79	74	83	9
	2 p.m.		80	77	86	9
	4 p.m.		78	79	86	7
May 28	6 p.m.	Clear	73	80	84	4
	8 p.m.		71	77	81	4
	10 p.m.		70	75	79	4
	12 midnight		70	74	76	2
	2 a.m.		69	71	75	4
	4 a.m.		70	69	73	4
	6 a.m.		69	68	72	4
	8 a.m.		77	68	74	6
	10 a.m.		79	75	80	5
	12 noon		80	79	88	9
	2 p.m.		80	82	89	7
	4 p.m.		77	82	87	5
	6 p.m.		74	81	84	3
	8 p.m.		72	77	81	4
	10 p.m.		70	75	78	3
	12 midnight		69	73	76	3

TABLE 3  
*Soil temperatures at Wahiawa—July 23 to 30, 1923*

DATE	HOURL	WEATHER	TEMPERATURE OF AIR	TEMPERATURE OF BARE SOIL	TEMPERATURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
July 23	2 a.m.	Clear	72	78	79	1
	4 a.m.		72	76	78	2
	6 a.m.		71	75	76	1
	8 a.m.		75	75	76	1
	10 a.m.		79	76	80	4
	12 noon		80	77	83	6
	2 p.m.		82	79	88	9
	4 p.m.		81	81	90	9
	6 p.m.		77	81	89	8
	8 p.m.		75	77	86	9
	10 p.m.		74	75	83	8
	12 midnight		73	74	81	7
July 24	2 a.m.	Clear	72	72	80	8
	4 a.m.		70	71	77	6
	6 a.m.		68	70	76	6
	8 a.m.		76	72	76	4
	10 a.m.		80	75	82	7
	12 noon		84	79	89	10
	2 p.m.		82	82	93	11
	4 p.m.		81	82	91	9
	6 p.m.		77	81	88	7
	8 p.m.		74	78	86	8
	10 p.m.		74	76	83	7
	12 midnight		74	73	82	9
July 25	2 a.m.		73	72	80	8
	4 a.m.		71	71	78	7
	6 a.m.		70	70	77	7
	8 a.m.		77	71	77	6
	10 a.m.		82	75	84	9
	12 noon		85	80	94	14
	2 p.m.		85	84	97	13
	4 p.m.		83	87	95	8
	6 p.m.		78	87	92	5
	8 p.m.		74	82	89	7
	10 p.m.		74	81	86	5
	12 midnight		74	77	84	7
July 26	2 a.m.	Clear	73	76	83	7
	4 a.m.		72	75	81	6
	6 a.m.		73	75	80	5
	8 a.m.		78	74	81	7
	10 a.m.		82	75	87	12
	12 noon		84	79	95	16
	2 p.m.		84	82	97	15
	4 p.m.		82	86	95	9
	6 p.m.		76	88	92	4

DATE	HOOR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
July 26	8 p.m.	Clear	73	86	89	3
	10 p.m.		71	82	86	4
	12 midnight		71	77	84	7
July 27	2 a.m.		70	76	82	6
	4 a.m.		69	74	80	6
	6 a.m.		70	73	79	6
	8 a.m.		77	71	80	9
	10 a.m.		82	72	89	17
	12 noon		84	78	97	19
	2 p.m.		84	82	98	16
	4 p.m.		80	86	96	10
	6 p.m.		77	90	93	3
	8 p.m.		75	86	90	4
	10 p.m.		74	82	87	5
	12 midnight		72	81	85	4
July 28	2 a.m.	Clear	72	79	83	4
	4 a.m.		70	77	82	5
	6 a.m.		72	76	80	4
	8 a.m.		76	74	81	7
	10 a.m.		79	74	84	10
	12 noon		82	75	90	15
	2 p.m.		81	80	92	12
	4 p.m.		77	81	90	9
	6 p.m.		74	83	88	5
	8 p.m.		73	81	85	4
	10 p.m.		72	80	83	3
	12 midnight		71	77	81	4
July 29	2 a.m.		70	76	80	4
	4 a.m.		69	74	78	4
	6 a.m.		72	73	77	4
	8 a.m.		78	72	79	7
	10 a.m.		80	72	85	13
	12 noon		82	75	90	15
	2 p.m.		81	77	92	15
	4 p.m.		78	81	90	9
	6 p.m.		74	82	88	6
	8 p.m.		72	82	85	3
	10 p.m.		72	81	83	2
	12 midnight		71	77	81	4
July 30	2 a.m.	Cloudy and rain	70	76	79	3
	4 a.m.		69	75	78	3
	6 a.m.		69	74	76	2
	8 a.m.		72	74	78	4
	10 a.m.		75	77	79	2
	12 noon		80	82	83	1

TABLE 4  
Soil temperatures at Wahiawa—October 18 to 28, 1923

DATE	HOOR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
October 18	2 p.m.		76	79	85	6
	4 p.m.		74	79	83	4
	6 p.m.		72	77	81	4
	8 p.m.		71	74	79	5
	10 p.m.		72	73	77	4
	12 midnight		71	72	76	4
October 19	2 a.m.	Clear	69	71	75	4
	4 a.m.		69	70	73	3
	6 a.m.		70	69	72	3
	8 a.m.		78	69	73	4
	10 a.m.		83	72	79	7
	12 noon		85	75	86	11
	2 p.m.	Rain	82	81	88	7
	4 p.m.		78	84	86	2
	6 p.m.		73	83	83	0
	8 p.m.		72	80	81	1
	10 p.m.		71	78	79	1
	12 midnight		70	76	77	1
October 20	2 a.m.	Clear	70	75	76	1
	4 a.m.		70	73	75	2
	6 a.m.		74	72	74	2
	8 a.m.		80	72	75	3
	10 a.m.		82	73	81	8
	12 noon		84	76	88	12
	2 p.m.	Rain	83	82	89	7
	4 p.m.		78	86	88	2
	6 p.m.		74	85	85	0
	8 p.m.		72	82	82	0
	10 p.m.		70	80	80	0
	12 midnight		70	77	78	1
October 21	2 a.m.		70	75	76	1
	4 a.m.		68	74	75	1
	6 a.m.		72	73	74	1
	8 a.m.		76	72	75	3
	10 a.m.		80	72	77	5
	12 noon		84	74	84	10
	2 p.m.	Rain	81	77	86	9
	4 p.m.		77	83	85	2
	6 p.m.		73	82	82	0
	8 p.m.		72	81	80	-1
	10 p.m.		71	79	78	-1
	12 midnight		70	75	76	1
October 22	2 a.m.	Rain	68	73	74	1
	4 a.m.		66	72	73	1
	6 a.m.		70	71	72	1

TABLE 4—Continued

DATE	HOUR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
October 22	8 a.m.		78	69	73	4
	10 a.m.		79	69	76	7
	12 noon		78	77	80	3
	2 p.m.		76	76	81	5
	4 p.m.		77	75	81	6
	6 p.m.		74	74	79	5
	8 p.m.		72	73	78	5
	10 p.m.		71	72	76	4
	12 midnight		70	71	75	4
October 23	2 a.m.		68	69	73	4
	4 a.m.		67	69	72	3
	6 a.m.		68	68	71	3
	8 a.m.		79	70	72	2
	10 a.m.		82	74	78	4
	12 noon		83	77	83	6
	2 p.m.		82	81	86	5
	4 p.m.		77	82	85	3
	6 p.m.		74	81	83	2
	8 p.m.	Showers	71	79	81	2
	10 p.m.		70	76	78	2
	12 midnight		69	74	77	3
October 24	2 a.m.		67	73	75	2
	4 a.m.		65	72	73	1
	6 a.m.		67	69	72	3
	8 a.m.		78	69	73	4
	10 a.m.		83	73	79	6
	12 noon		84	77	86	9
	2 p.m.	Cloudy	83	85	88	3
	4 p.m.		79	85	87	2
	6 p.m.		74	83	84	1
	8 p.m.		72	80	82	2
	10 p.m.		70	78	79	1
	12 midnight		69	75	78	3
October 25	2 a.m.		70	74	76	2
	4 a.m.		69	73	75	2
	6 a.m.		71	72	74	2
	8 a.m.		75	72	76	4
	10 a.m.		80	74	77	3
	12 noon		72	75	79	4
	2 p.m.		77	76	81	5
	4 p.m.	Rain	75	77	81	4
	6 p.m.		72	76	79	3
	8 p.m.		70	74	77	3
	10 p.m.		69	73	75	2
	12 midnight		67	72	74	2

TABLE 4—*Continued*

DATE	HOOR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
October 26	2 a.m.	Clear	66	71	73	2
	4 a.m.		65	70	71	1
	6 a.m.		65	69	70	1
	8 a.m.		73	67	70	3
	10 a.m.		80	69	73	4
	12 noon		83	73	80	7
	2 p.m.		81	77	85	8
	4 p.m.		79	80	85	5
	6 p.m.		74	80	83	3
	8 p.m.		71	78	80	2
	10 p.m.		71	75	78	3
	12 midnight		71	74	76	2
October 27	2 a.m.		69	73	74	1
	4 a.m.		69	72	73	1
	6 a.m.		70	71	72	1
	8 a.m.		77	70	71	1
	10 a.m.		79	70	75	5
	12 noon		82	72	80	8
	2 p.m.		81	77	84	7
	4 p.m.		79	81	85	4
	6 p.m.		75	82	83	1
	8 p.m.		73	79	81	2
	10 p.m.		73	77	79	2
	12 midnight		72	75	77	2
October 28	2 a.m.		72	74	75	1
	4 a.m.		72	73	74	1
	6 a.m.		71	72	73	1
	8 a.m.		75	71	73	2
	10 a.m.		75	71	74	3
	12 noon		78	73	76	3

ture of the day became somewhat higher, the nights were also appreciably warmer. This resulted in a greater difference in soil temperature between the mulched and unmulched plots, both in the daytime and at night. This is shown in table 3 for the last week in July. The same relative conditions prevailed during August and September. In October, broken showery weather was prevalent and the effect of this condition upon the soil temperatures is shown in table 4, for the period from October 18 to 28. During this time, there were frequent changes from bright sunshine to clouds, showers and rain.

The effect of weather upon the relative soil temperatures is more clearly shown during a rainy period in early December. The difference in temperature between the paper mulched plots and those with bare soil largely disappeared during the periods of heaviest precipitation, and occasional negative figures are shown in the column of differences. With sunshiny weather the



TABLE 5  
*Soil temperatures at Wahiawa—rainy weather November 30 to December 9, 1923*

DATE	HOOR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
November 30	2 a.m.		64	68	69	1
	4 a.m.		63	67	68	1
	6 a.m.		65	66	67	1
	8 a.m.		71	66	67	1
	10 a.m.		77	67	69	2
	12 noon		80	70	74	4
	2 p.m.		80	74	78	4
	4 p.m.		78	77	79	2
	6 p.m.		72	78	77	-1
	8 p.m.		68	75	75	0
	10 p.m.		65	73	73	0
December 1	12 midnight	Heavy rains	61	70	71	1
	2 a.m.		64	68	69	1
	4 a.m.		63	67	68	1
	6 a.m.		63	66	67	1
	8 a.m.		70	66	67	1
	10 a.m.		73	67	68	1
	12 noon		76	68	70	2
	2 p.m.		78	71	73	2
	4 p.m.		77	73	75	2
	6 p.m.		71	74	74	0
	8 p.m.		68	73	72	-1
December 2	10 p.m.	Rain	68	71	71	0
	12 midnight		68	69	70	1
	2 a.m.		68	68	68	0
	4 a.m.		67	67	67	0
	6 a.m.		65	67	66	-1
	8 a.m.		70	66	67	1
	10 a.m.		79	66	69	3
	12 noon		81	68	73	5
	2 p.m.		82	73	78	5
	4 p.m.		78	76	79	3
	6 p.m.		73	78	77	-1
December 3	8 p.m.	Rain	72	77	75	-2
	10 p.m.		71	74	73	-1
	12 midnight		70	73	72	-1
	2 a.m.		68	72	70	-2
	4 a.m.		68	70	69	-1
	6 a.m.		65	68	68	0
	8 a.m.		73	67	68	1
	10 a.m.		80	76	71	-5
	12 noon		81	77	76	-1
	2 p.m.		78	76	79	3
	4 p.m.	Clear	76	74	79	5
	6 p.m.		72	73	77	4
	8 p.m.		72	72	76	4

TABLE 5—Continued

DATE	HOOR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
December 3	10 p.m.		71	70	74	4
	12 midnight		70	70	72	2
December 4	2 a.m.		69	69	71	2
	4 a.m.		68	68	71	3
	6 a.m.		69	68	70	2
	8 a.m.		71	72	70	-2
	10 a.m.	Showers	76	73	71	-2
	12 noon		77	74	75	1
	2 p.m.		77	75	77	2
	4 p.m.		75	74	78	4
	6 p.m.		72	73	77	4
	8 p.m.	Clear	71	72	75	3
	10 p.m.		71	71	74	3
	12 midnight		70	69	73	4
December 5	2 a.m.		70	68	72	4
	4 a.m.		70	68	71	3
	6 a.m.		69	67	70	3
	8 a.m.		73	68	69	1
	10 a.m.		74	72	71	-1
	12 noon	Showers	76	73	73	0
	2 p.m.		76	73	75	2
	4 p.m.		74	72	75	3
	6 p.m.		71	71	74	3
	8 p.m.		70	69	72	3
	10 p.m.	Cloudy	69	68	71	3
	12 midnight		68	68	70	2
December 6	2 a.m.		68	67	70	3
	4 a.m.		67	66	69	3
	6 a.m.		68	66	68	2
	8 a.m.		69	67	68	1
	10 a.m.		73	69	70	1
	12 noon		75	73	72	-1
	2 p.m.	Showers	75	74	74	0
	4 p.m.		72	73	75	2
	6 p.m.		70	72	73	1
	8 p.m.		69	71	72	1
	10 p.m.		69	69	71	2
	12 midnight		69	68	70	2
December 7	2 a.m.		68	67	69	2
	4 a.m.		68	66	68	2
	6 a.m.		69	66	67	1
	8 a.m.		76	68	68	0
	10 a.m.		77	70	71	1
	12 noon		78	72	75	3
	2 p.m.		77	74	78	4

TABLE 5—*Continued*

DATE	HOOR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
December 7	4 p.m.	Clear	73	74	78	4
	6 p.m.		72	72	76	4
	8 p.m.		70	70	74	4
	10 p.m.		69	69	73	4
	12 midnight		67	67	71	4
December 8	2 a.m.	Clear	65	66	69	3
	4 a.m.		64	65	68	3
	6 a.m.		64	66	67	1
	8 a.m.		74	67	68	1
	10 a.m.		79	68	71	3
	12 noon		80	72	75	3
	2 p.m.		79	75	78	3
	4 p.m.		75	75	79	4
	6 p.m.		69	73	76	3
	8 p.m.		69	71	74	3
	10 p.m.		69	69	72	3
	12 midnight		68	68	72	4
	2 a.m.		68	68	71	3
December 9	4 a.m.	Clear	67	67	70	3
	6 a.m.		67	67	69	2
	8 a.m.		73	68	70	2
	10 a.m.		78	71	75	4
	12 noon		81	73	79	6
	2 p.m.		80	76	83	7
	4 p.m.		74	78	82	4
	6 p.m.		70	76	79	3
	8 p.m.		68	74	76	2
	10 p.m.		68	72	74	2
	12 midnight		66	70	73	3

temperature rapidly rose in the paper mulched plots. The temperature differences in clear weather were approximately the same as those previously found during the spring months, that is, the paper plots were 4 to 5° warmer than those with bare soil during the early afternoon. The temperature differences during the night ranged from 2 to 3° during clear weather.

During the spring months the same general conditions prevailed. In clear weather the paper mulched plots were continuously higher in temperature than the plots of bare soil. During periods of comparative freedom from high wind, the differences between the mulched and unmulched plots were surprisingly constant, both during the day and night. Such a period of calm, clear weather from January 31 to February 7, 1924, is shown in table 6.

On May 1, 1924, the soil in the mulched and unmulched plots was thoroughly cultivated and pineapple plants were put in. Up to January 1, 1925, as the plants had not appreciably shaded over the plots, the temperature rela-

TABLE 6

*Soil temperatures at Wahiawa—calm clear weather, January 31 to February 7, 1924*

DATE	HOUR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
January 31	2 p.m.	Clear	77	74	80	6
	4 p.m.		74	73	79	6
	6 p.m.		70	70	77	7
	8 p.m.		66	68	74	6
	10 p.m.		62	66	72	6
February 1	12 midnight	Clear	59	64	69	5
	2 a.m.		58	62	67	5
	4 a.m.		58	61	66	5
	6 a.m.		60	60	65	5
	8 a.m.		76	60	66	6
	10 a.m.	Clear	80	63	71	8
	12 noon		80	68	77	9
	2 p.m.		79	74	81	7
	4 p.m.		77	75	81	6
	6 p.m.		69	73	79	6
	8 p.m.	Clear	65	70	75	5
	10 p.m.		62	67	73	6
	12 midnight		65	66	71	5
	2 a.m.		66	65	70	5
	4 a.m.		66	65	69	4
February 2	6 a.m.	Clear	69	64	68	4
	8 a.m.		75	63	70	7
	10 a.m.		78	65	74	9
	12 noon		79	68	78	10
	2 p.m.		81	72	82	10
	4 p.m.	Clear	76	75	82	7
	6 p.m.		69	74	79	5
	8 p.m.		64	71	76	5
	10 p.m.		63	68	73	5
	12 midnight		63	66	71	5
February 3	2 a.m.	Clear	62	65	69	4
	4 a.m.		61	64	68	4
	6 a.m.		61	63	67	4
	8 a.m.		72	63	68	5
	10 a.m.		79	64	72	8
	12 noon	Clear	79	68	76	8
	2 p.m.		80	72	78	6
	4 p.m.		75	73	78	5
	6 p.m.		67	73	76	3
	8 p.m.		65	70	73	3
	10 p.m.	Clear	63	67	71	4
	12 midnight		63	66	69	3
	2 a.m.		64	65	68	3
	4 a.m.		64	64	68	4
	6 a.m.		66	63	67	4

\* Week ending February 10, "Good weather all week; no rain but Kona wind and clouds."

TABLE 6—Continued

DATE	HOOR	WEATHER	TEMPERA- TURE OF AIR	TEMPERA- TURE OF BARE SOIL	TEMPERA- TURE OF SOIL UNDER PAPER	DIFFERENCE DUE TO PAPER
			°F.	°F.	°F.	°F.
February 4	8 a.m.	Clear	70	63	68	5
	10 a.m.		73	66	70	4
	12 noon		73	68	70	2
	2 p.m.		77	70	73	3
	4 p.m.		76	70	76	6
	6 p.m.		70	67	75	8
	8 p.m.		66	64	73	9
	10 p.m.		63	63	70	7
	12 midnight		60	62	68	6
February 5	2 a.m.	Clear	61	61	67	6
	4 a.m.		61	60	66	6
	6 a.m.		62	60	65	5
	8 a.m.		69	62	65	3
	10 a.m.		77	64	67	3
	12 noon		78	68	73	5
	2 p.m.		78	72	77	5
	4 p.m.		75	72	78	6
	6 p.m.		70	70	76	6
	8 p.m.		69	67	74	7
	10 p.m.		68	66	72	6
	12 midnight		68	65	71	6
February 6	2 a.m.	Clear	65	64	69	5
	4 a.m.		62	63	68	5
	6 a.m.		59	62	67	5
	8 a.m.		66	60	66	6
	10 a.m.		72	63	68	5
	12 noon		77	68	73	5
	2 p.m.		77	72	78	6
	4 p.m.		75	73	80	7
	6 p.m.		69	72	78	6
	8 p.m.		63	69	75	6
	10 p.m.		62	65	72	7
	12 midnight		64	64	70	6
	2 a.m.		63	64	69	5
	4 a.m.		64	63	68	5
	6 a.m.		65	63	67	4
February 7	8 a.m.	Clear	71	63	68	5
	10 a.m.		75	64	70	6
	12 noon		77	65	74	9
	2 p.m.		79	70	78	8
	4 p.m.		75	73	79	6
	6 p.m.		69	73	77	4
	8 p.m.		68	70	75	5
	10 p.m.		66	67	73	6
	12 midnight		63	66	71	5

tionships between the mulched and unmulched soils have continued to show the same differences on the paper and no paper soils. As this report closes with January, 1925, a longer period of time will be required to find whether the effect of the plants in shading the soil will greatly reduce the temperature differences between the mulched and unmulched plots.

*Effect of paper mulch on moisture retention, ammonification, and nitrification*

In order to study the effect of the paper mulch upon moisture retention and upon the development of ammonia and nitrates under field conditions, a series of plots was laid out at Wahiawa in May, 1924. Half of the plots were mulched with a heavy grade of asphalt impregnated and coated paper the same as that which had been used in the studies of soil temperature. One series of mulched and unmulched plots was untreated; a second series received ammonium sulfate at the rate of 1000 pounds per acre, supplying 208 pounds of nitrogen; a third series received mixed fertilizer at the rate of 1000 pounds per acre. The mixed fertilizer contained  $11\frac{1}{2}$  per cent total nitrogen, of which 7.5 per cent was from ammonium sulfate and 4.0 per cent from dried blood. The mixed fertilizer also supplied  $6\frac{1}{2}$  per cent phosphoric acid and 5 per cent potash. The plots were sampled after the soil was thoroughly prepared, the fertilizer was then worked in, the paper mulch was applied to the mulched plots, and the pineapple plants were put in. The first set of samples on May 9 at the beginning of the graphs therefore show the condition of the soil before it had received any treatment other than cultivation. Three sets of composite samples were collected from each plot. The figures plotted in the accompanying graphs are the average of the results obtained on the three composites collected from each plot. The subsamples of the composites analyzed were all taken to a depth of 1 foot.

The observations were continued from May 9, 1924, to December 11, 1924. This period of time covered a wide range of weather conditions and included the warmest summer months and the early winter season.

In order to make the figures obtained upon the various plots available for ready comparison, the data for moisture, nitrate nitrogen, and ammonia nitrogen are shown in the form of graphs. It may be stated that the results obtained upon the composite samples for moisture and nitrate nitrogen showed a significantly close agreement. The consistent course of the curves at all sampling periods is further evidence of the significance of the figures obtained. This was not the case with the figures for ammonia nitrogen. Here a very high variability was encountered; differences of over 100 per cent between composite samples were not unusual. The graphs for ammonia nitrogen are included; these also demonstrate that there is no consistent difference from which definite conclusions can be drawn.

It should be pointed out that the differences found in the soils of the paper and no paper plots will express only part of the differences caused by the paper

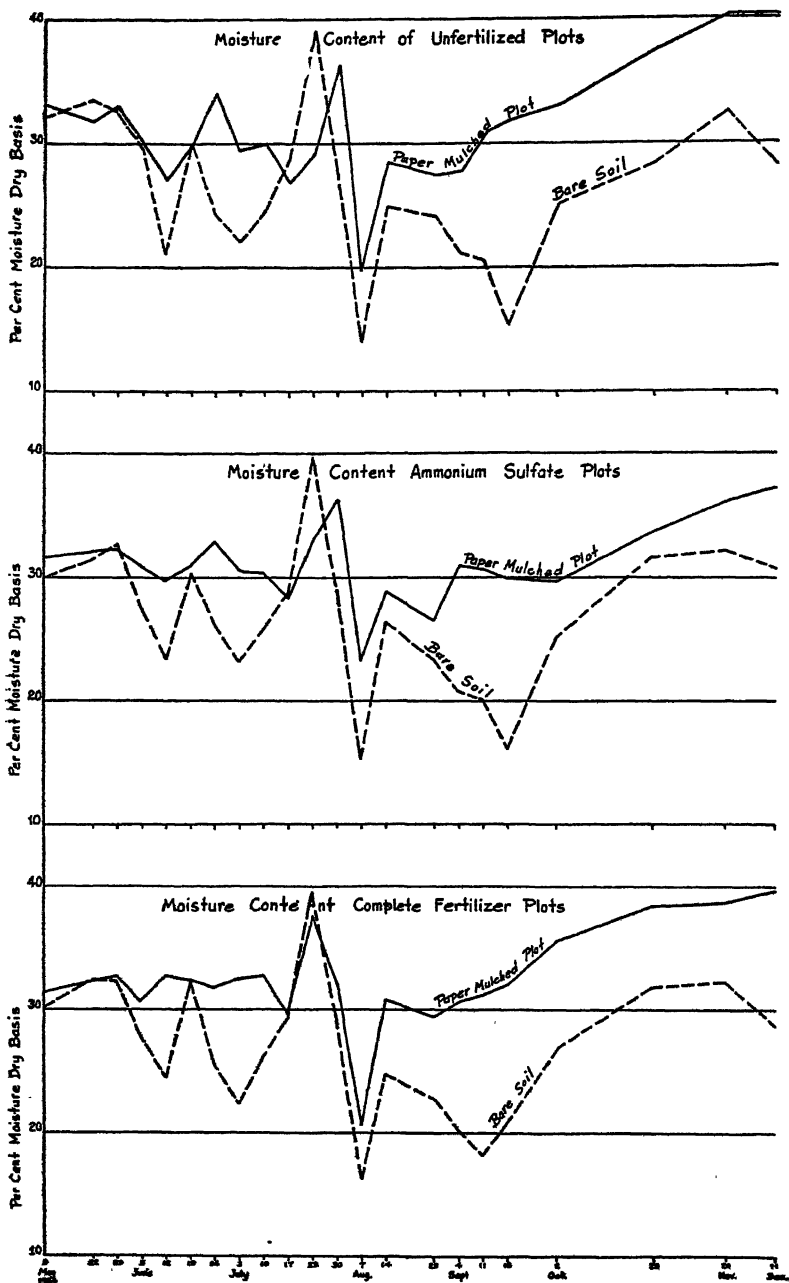


FIG. 2. MOISTURE CONTENT OF PAPER-MULCHED AND BARE SOIL PLOTS DURING THE FIRST 7 MONTHS' GROWTH

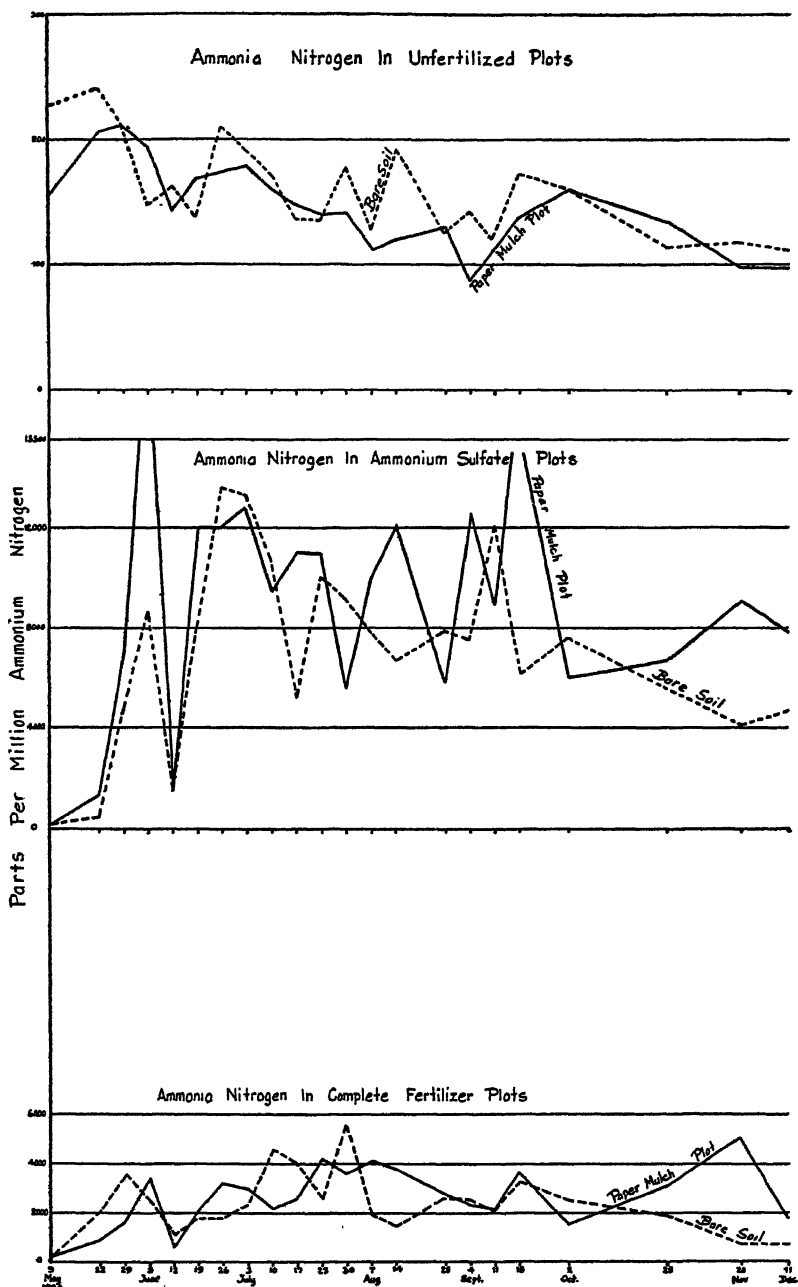


FIG. 3. AMMONIA NITROGEN CONTENT OF PAPER-MULCHED AND BARE SOIL PLOTS DURING THE FIRST 7 MONTHS' GROWTH



mulch. The pineapple plants themselves have been found by the authors in other work (3) to be 30 to 40 per cent larger by weight when grown under the paper mulch than in bare soil. This difference represents a notable extraction of moisture and nutrients from the soil. The authors are justified, there-

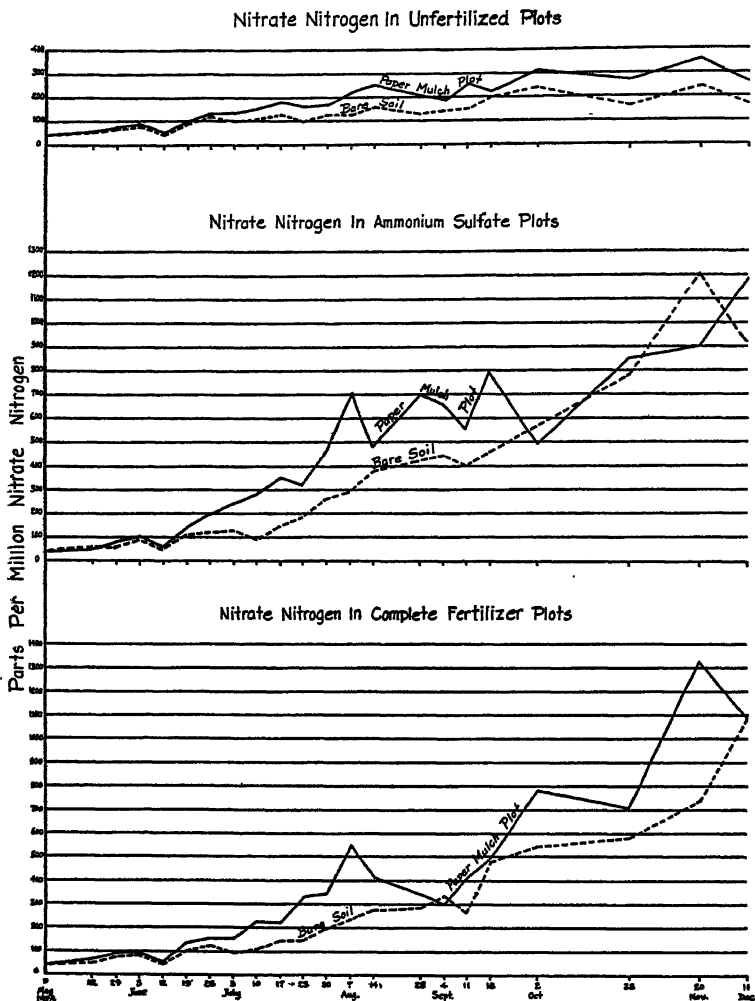


FIG. 4. NITRATE NITROGEN CONTENT OF PAPER-MULCHED AND BARE SOIL PLOTS DURING THE FIRST 7 MONTHS' GROWTH

fore, in believing that any balance of higher moisture or nutrients in the paper mulched soils would be larger still if allowance were made for the amounts of moisture and nutrients which go to nourish the larger plants.

The following analytical methods were employed. Moisture was deter-

mined by drying 100-gm. portions of soil over night at 100° to 103°C. Nitrates were determined by the phenoldisulfonic acid method on a portion of a 1 to 5 water extract. For the determination of ammonia nitrogen, an extensive study was made of a considerable number of aeration methods which have been proposed for the determination of ammonia in soils. These methods are essentially modifications of Folin's (1) aeration method for the determination of ammonia in physiological solutions. The Hawaiian soils have a large content of colloidal material consisting mainly of the hydrated oxides of iron and aluminum. These colloids have a high fixing power for ammonia and other radicals. We did not find it possible to recover by any of the aeration methods more than 40 to 50 per cent of the ammonia added to Wahiawa soil as ammonium sulfate. We were finally reduced to determining the ammonia in our samples by boiling 100 gm. of soil in a copper flask with 250 cc. of water and 5 gm. of magnesium oxide, the distillate being passed into standard acid. This method gave recoveries of approximately 90 per cent of the added ammonia, but we are, of course, well aware of the errors introduced by this method through the action of the magnesium oxide on the soil organic matter.

The results of the moisture determinations are presented in figure 2. The graphs show a consistently higher moisture content in the paper mulched soil. The only sampling period when this was not the case was on July 23, when rain fell just as the sampling of the plots was started. The results for this date show the rapid absorption of the rain in the bare soil, whereas the moisture had not had a chance to move away from the plants and borders of the plot in the paper mulched plot. Most of the moisture probably gains entrance to the paper mulched soil by the holes around the plants. Their leaves serve as excellent water collectors during rains or showers and conduct it down to the basal roots and so into the soil.

The figures for nitrate nitrogen are given in figure 3. Here again there was a consistently higher nitrate nitrogen content in all plots which were under the paper mulch.

The data on ammonia nitrogen are given in figure 4, but because of the variability previously noted in the figures obtained on the composite samples, the fluctuating content of ammonia nitrogen cannot be considered significant. It would require a special study to determine whether significant figures for this constituent could even be obtained by a large increase in the number of subsamples.

#### CONCLUSIONS

1. The foregoing results would appear to warrant the conclusion that the paper mulch exerts several effects upon Hawaiian soils planted to pineapples. Probably chief among these effects are a higher soil temperature, a higher content of soil moisture, and a more rapid elaboration of soil nutrients.

2. The temperature effect of the paper mulch varied with the weather and

with the season of the year, and will probably also vary with the degree of shading of the mulch paper by the pineapple plants.

3. The greatest increase in soil temperature due to mulching paper occurred in clear, bright weather. Showers and rain decreased the temperature differences between the mulched and the bare soil. In heavy rains the temperature differences disappeared and for short periods the bare soil appeared to be slightly warmer.

4. The maximum soil temperature generally occurred at 2:00 to 4:00 p.m., about 2 hours after the maximum air temperature.

5. The greatest differences between mulched and bare soils occurred during the warmest months of the year—in July, August, and September. These maximum temperature differences frequently amounted to 12 or 15°F. in the afternoon and decreased to about 4 or 5° in the night. During the winter months the maximum daytime differences in temperature generally ranged from 5 to 8°F. and decreased to 2 to 4° at night.

6. The effect of the paper mulch upon the retention of moisture in field soils was found to be appreciable. The paper mulched soils had a notably higher moisture content than the bare land.

7. A consistently higher nitrate content was found in paper mulched soils which had received no fertilizer as well as in those receiving ammonium sulfate and a complete mixture.

8. This higher nitrate content of paper mulched soils is taken to indicate that the paper mulch probably causes a more rapid elaboration of the principal soil nutrients in mulched soils.

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- (2) LARSEN, L. D. 1917 Paper mulches for weed control. *Planters' Rec.* 17: 123-133.
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#### PLATE 1

FIG. 1. Method of laying the paper mulch.

FIG. 2. Newly planted field of pineapples, Experiment Station of Hawaiian Pineapple Cannery Association.

G. R. STEWART, E. C. THOMAS AND JOHN HORNER



FIG. 1



FIG. 2



# RESIDUAL EFFECTS OF FORTY YEARS CONTINUOUS MANURIAL TREATMENT: III. ULTIMATE FATE AND SOME PHYSICAL AND CHEMICAL EFFECTS OF APPLIED LIME

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Data secured as the result of detailed studies of the plots which have received lime both as pulverized limestone and as burned lime show that in each case the limed soils at the end of 40 years of treatment contain more organic matter and nitrogen than the corresponding unlimed soils (8). The plot treated with limestone produced a total of 9560 pounds of dry matter in excess of that produced by the plot receiving the burned lime treatment. The soil treated with burned lime, however, contained 1087 pounds organic matter in excess of the limestone soil. The second paper of this series (11) shows that a close relationship exists between the total yields and the residual organic matter of similarly treated plots. On the basis of this established relationship it is computed that the limestone treatment has caused the decomposition of 9862 pounds of organic matter in excess of the untreated soil as compared to 4157 pounds where burned lime was used. In other words, bacterial action has been greater in the case of the limestone treatment. The difference in the effect of the two forms of lime is attributed to the variation in the actual amounts of available CaO applied and not to chemical differences between the two materials. The excess of available CaO applied as burned lime has apparently depressed bacterial action as compared to the coarse limestone treatment tending to reduce rather than stimulate the decay and ultimate loss of soil organic matter.

This paper deals with a study of the fate of the two forms of lime including the relative decomposition of the different grades of limestone particles. The results of certain laboratory studies, designed to show the effect of excess of lime on bacterial activity as measured by the evolution of  $\text{CO}_2$  are also included. The data secured by Brown, MacIntire, and others as the results of earlier studies of these limed plots, dealing with the effect of lime on the physical and chemical properties of the soils, are presented and discussed.

## BURNED LIME AND LIMESTONE TREATMENTS

Since 1881 burned lime has been applied both to the unfertilized soil and to the soil treated biennially with 6 tons of barnyard manure. Limestone has been applied only to the unfertilized soil. The burned lime has been applied

to each corn crop at the rate of 2 tons per acre. The limestone has been applied both to the corn and to the wheat crop at the rate of 2 tons per acre. From 1881 to 1921, therefore, 20 tons of burned lime and 40 tons of limestone have been applied. From 1881 to 1910 the burned lime was slaked in piles before spreading. Since 1910 raw ground lime has been used. During the first 27 years (1881-1908) very coarse limestone was applied, only 36.81 per cent of which would pass a 0.5-mm. screen. Since 1908 a much finer product has been used. Chemical examination of several composite samples of the lime shows that the burned lime was 88 per cent and the limestone 93.2 per cent pure. On the basis of the chemical composition the acre equivalent of 35,200 pounds of CaO was applied as burned lime and 41,754 pounds CaO as limestone.

The samples of soil used for the present study were taken from the west half of tier 4. This area was selected because the plots are level, thus avoiding any error due to surface washing. Samples were taken from both the surface (0-7 inches) and subsurface (7-14 inches).

TABLE 1  
*Mechanical composition of limestone used on plot 34 from 1881 to 1921*

	1881- 1908	1909- 1915	1916- 1917	1918- 1921	1881- 1921 AVER- AGE	ACTUAL WEIGHT OF LIME- STONE	EQUIVA- LENT WEIGHT OF CaCO <sub>3</sub> *
	Number of applications						
	14	3	1	2	20		
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>pounds</i>	<i>pounds</i>
Between 4 and 1 mm.....	48.34	0	2.50	16.00	35.56	28,450	26,515
Between 1 and 0.5 mm.....	14.85	0	4.00	22.40	12.83	10,268	9,590
Between 0.5 and 0.25 mm.....	16.15	11.38	5.50	30.10	16.31	13,038	12,151
Pass 0.25 mm.....	20.66	88.62	88.00	31.50	35.30	28,244	26,304
Total.....	100	100	100	100	100	80,000	74,560

\* 93.2 per cent CaCO<sub>3</sub>.

#### MECHANICAL ANALYSIS OF LIMESTONE USED FROM 1881 TO 1921

Four different grades of limestone were used during the progress of the experiment as shown in table 1. Of the limestone used (1881-1908) 70 per cent was very coarse only 20.66 per cent of which would pass a 0.25-mm. screen. Column 5 shows the approximate mechanical analysis for the entire period.

#### RECOVERY OF RESIDUAL LIMESTONE PARTICLES

The plot soil used for mechanical and chemical analysis represented the material that passed a 4-mm. screen instead of a 1-mm. screen as commonly used. The use of a coarse screen was necessary to avoid discarding the coarser limestone particles. The limestone treated soil (plot 34) and the nearest check

plot (plot 36) were then submitted to mechanical analysis using the soil that passed the 4-mm. screen. For several hours 400 gm. of soil were shaken with distilled water to which were added a few drops of ammonia. The material was then transferred to the 4-mm. screen to which were attached the several other screens in the order shown. The several separates were again washed, dried at 100°C., and weighed. The weight per acre of each mechanical separate was then computed on the basis of the weight of 1 mm. soil taken as 2,000,000 pounds per acre. The separates of both the limestone treated and the untreated soils were then finely ground and analyzed for total carbonates. The carbonates in excess of the untreated check soil are reported as residual limestone. Table 2 shows the proportions of recovered limestone in relation to the approximate amount applied from 1881-1921.

It is evident from a study of table 2 that the coarse limestone particles have undergone considerable change in their mechanical relationship. Of the 26,515

TABLE 2  
*Calcium carbonate applied to plot 34 and that recovered after forty years*

	ACTUAL CaCO <sub>3</sub> APPLIED	WEIGHT OF SOIL PER ACRE		CaCO <sub>3</sub> RECOVERED		TOTAL CaCO <sub>3</sub> RECOVERED
		Surface 0-7 inches	Sub-surface 7-14 inches	Surface 0-7 inches	Sub-surface 7- 14 inches	
	pounds	pounds	pounds	pounds	pounds	pounds
Between 4 and 1 mm. ....	26,515	113,200	113,200	11,501	797	12,298
Between 1 and 0.5 mm. ....	9,590	51,139	46,702	8,642	610	9,252
Between 0.5 and 0.25 mm. ....	12,151	143,125	115,915	25,047	1,462	26,509
Pass 0.25 mm. ....	26,304	1,805,726	1,837,383	8,062*	808*	8,870
Total. ....	74,560	2,113,200	2,113,200	53,252	3,677	56,929

\* By difference.

pounds that originally failed to pass a 1-mm. screen only 12,298 pounds remain. The increase in the proportion of particles between 0.5 and 0.25 mm. shows that these coarse materials have been reduced in size sufficiently to enable them to pass the 0.5-mm. screen. Of the original material that failed to pass the 0.25-mm. (100-mesh) screen 99.6 per cent was recovered. During the 40 years 17,631 pounds of carbonates have undergone decomposition. This loss of carbonates has been confined for the most part to the material that originally passed the 0.25-mm. screen. Of the 26,304 pounds of fine material applied, only 8870 pounds were recovered equivalent to a loss of 17,434 pounds. It is recognized that such a study can only approximate the truth and was undertaken with the hope of adding to our knowledge concerning the value of limestone of different degrees of fineness supplementary to previous studies (7).



## FATE OF BURNED LIME AND LIMESTONE

Total CaO and inorganic CO<sub>2</sub> were determined on both the surface and sub-surface samples representative of plots treated with burned lime and limestone. The same determinations were also made on the nearest untreated plots including also the plot treated with 6 tons of manure. The results secured are shown in table 3. The CaO and CO<sub>2</sub> results are corrected for that found in the unlimed plot 16 (manure), and check plots 24 and 36. Reference to table 3 shows that 87 per cent of the CaO applied in limestone remained in the surface soil at the end of 40 years as compared to 61 and 63 per cent in case of burned

TABLE 3  
*CaO found in the soil at the end of forty years*

	1881-1921		
	Plot 22 (lime and manure)	Plot 23 (lime)	Plot 34 (limestone)
Total CaO applied (1881-1921) pounds per acre.....	35,200	35,200	41,754
*CaO recovered in surface soil (0-7 inches), per cent.....	1.005	1.010	1.720
Equivalent pounds per acre CaO.....	22,294	21,343	36,347
Total CaO lost from surface soil, pounds per acre.....	12,906	13,857	5,407
Per cent CaO lost of that applied.....	37	39	13
CaO recovered in subsurface* (7-14 inches) pounds per acre.....	7,715	9,620	3,487
CaO removed in crops, pounds per acre.....	836	502	550
CaO lost by drainage (by difference) pounds per acre.....	4,355	3,735	1,370
Inorganic CO <sub>2</sub> , (surface soil) per cent.....	0.535	0.545	1.110
Equivalent to CaCO <sub>3</sub> , pounds per acre.....	25,570	26,204	53,252
Per cent of CaO found present as CaCO <sub>3</sub> .....	64	69	82
CaCO <sub>3</sub> decomposed, pounds per acre.....	.....	.....	17,631
Average annual decomposition of CaCO <sub>3</sub> .....	.....	.....	441

\* In excess of unlimed soil.

lime. The manure applied to plot 22 had apparently little effect on the loss or retention of lime as compared to plot 23 where lime was used alone. The lime found in the subsurface represented for the most part that which was carried down mechanically by the variation in depths of plowing. Examination of the subsurface soil by means of a strong magnifying glass disclosed particles of limestone and carbonated granules of burned lime not found in the unlimed soils. From 1881 to 1921 only 4045 pounds CaO were lost by drainage as an average of the two burned lime applications compared to 1370 pounds CaO applied as limestone. Of the total CaO found on the burned lime plot only 66.5 per cent was present as CaCO<sub>3</sub> as compared to 82 per cent where limestone was used. The computed loss of lime by drainage appears lower than would be expected. The average annual decomposition of CaCO<sub>3</sub> is approximately the same as that found on DeKalb soil (9). In previous studies

of this nature the loss of lime from the normal plowed surface has been attributed to solution and subsequent drainage, no corrections being made for that carried down mechanically by the plow and deposited in the lower stratum below the normal or average plowed depth. Thus on plot 23, 9620 pounds of CaO were found at a depth of from nine to twelve inches as the result of unusually deep plowing during the progress of the experiment. This amount is equivalent to 69 per cent of the total removed from the surface seven inches. In comparing the computed loss of lime by drainage from this soil with the results of other studies it should be borne in mind that the soil here is frozen for the major part of the non-growing seasons. The average air temperature from October to March is 34.33°F. The soil is a heavy limestone silt and clay loam not subject to excessive leaching. The excessive amounts of lime applied to this soil, perhaps alkaline at the beginning of the experiment, would greatly reduce the amount of carbonates decomposed as compared to smaller applica-

TABLE 4

*Rate of limestone decomposition of soil from limestone treated plot compared with soils from check plots and plot treated with sulfate of ammonia*

SOURCE OF SOIL USED	PLOT 32	PLOT 34	PLOT 36
40 year treatment.....	Sulfate of ammonia	Limestone	Untreated
Veitch lime requirement (CaCO <sub>3</sub> ).....	5733	Alkaline	2525
Laboratory treatment (pounds per acre).....	3 tons CaCO <sub>3</sub>	3 tons CaCO <sub>3</sub>	3 tons CaCO <sub>3</sub>
Milligrams total CO <sub>2</sub> received in 30 days.....	1500.5	476.6	1024.8
Milligrams organic CO <sub>2</sub> .....	976.4	471.4	802.5
Milligrams inorganic CO <sub>2</sub> .....	524.1	6.2	222.3
Equivalent pounds per acre CaCO <sub>3</sub> .....	5954	69	2525

tions on an originally acid soil. MacIntire (5) in his study of the same plots in 1911 reports a considerably greater proportionate loss of lime. He, however, apparently discarded the soil material coarser than 1 mm. and also assumed that the lime used was chemically pure.

To determine the rate of limestone decomposition on a heavily limed soil as compared to soils low in lime content, a laboratory experiment was conducted in which the equivalent of 6 tons of limestone was applied to 450 gm. of soils from the heavily limed plot 34 and to soils from the untreated plots and those that have received sulfate of ammonia. The soils were kept at optimum moisture content for 30 days in large filtering flasks to which were attached CO<sub>2</sub> trains. At regular intervals the CO<sub>2</sub> evolved was absorbed in weighed soda lime tubes in the usual way. Table 4 shows the total milligrams of CO<sub>2</sub> collected from each soil. From the known organic and inorganic CO<sub>2</sub> content of the original treated soils the proportions of the CO<sub>2</sub> derived from the two sources are computed. The data included in table 4 show that the rate of limestone decomposition on the heavily limed soil was only 1 per cent of that on

the sulfate of ammonia treated soil and less than 3 per cent of that on the check plot soil. The decomposition of soil organic matter also proceeded more slowly on the limestone plot soil.

### *Chemical and Physical Effects of Lime*

*The effect on soil reaction.* Hydrogen-ion studies were made on each of the limed plots of the four tiers by the electrometric method. Since the burned

TABLE 5  
*Effect of burned lime and limestone on soil reaction*

	PLOT 22 (LIME AND MANURE)				PLOT 23 (LIME)				PLOT 34 (LIMESTONE)			
	Tier 1	Tier 2	Tier 3	Tier 4	Tier 1	Tier 2	Tier 3	Tier 4	Tier 1	Tier 2	Tier 3	Tier 4
Number months since last lime application.....	15	3	39	27	15	3	39	27	15	3	23	11
Hydrogen-ion concentration...	7.76	7.91	7.81	7.85	7.89	7.96	7.95	7.89	7.79	7.66	7.63	7.64

TABLE 6  
*Water-soluble nitrates and organic matter recovered on burned lime plot 23 and check plot 24*  
(Parts per million)

	TIER 1		TIER 2		TIER 3		TIER 4	
	Plowed oats stubble		Corn		Grass sod		Wheat stubble	
	Plot 23	Plot 24	Plot 23	Plot 24*	Plot 23	Plot 24	Plot 23	Plot 24†
Nitrates (NO <sub>3</sub> ).....	47.0	34.2	32.8	49.8	16.1	3.7	17.0	8.6
Total solids.....	378	195	380	260	325	150	420	200
Volatile matter.....	200	105	199	145	160	80	230	95
Inorganic CO <sub>2</sub> (in dry residue).....	38.8	20.0	45.0	15.0	33.8	2.0	26.3	1.0
Equivalent CaCO <sub>3</sub> .....	88.2	45.4	102.3	34.1	76.8	4.5	59.8	2.3
Organic CO <sub>2</sub> (in dry residue).....	135.0	67.7	77.5	43.0	85.0	66.3	86.3	65.0
Equivalent organic matter.....	63.4	31.8	36.4	20.2	39.9	31.2	40.6	30.9

\* Received 2 tons limestone 1922.

† Received 3 tons limestone 1923.

lime was applied only to the corn ground it was possible to study the progressive changes in soil reaction in relation to the length of time between lime applications. Table 5 shows the data secured as the result of these studies. These data show that the soil 3 months after the CaO was applied has practically the same OH-ion concentration as the soil to which the CaO had been applied 39 months previous. The two burned lime treatments show an average OH-ion concentration of 7.87 as compared to 7.68 on the limestone treated soil. The manure treatment has had no significant effect on the soil reaction. These

data are contrary to the results secured by Hoagland and Christie (4) who report the long duration of a high OH-ion concentration on soils treated with caustic lime.

*Effect of lime on water-soluble nutrients.* Plots 23 (CaO) and 24 (check)<sup>1</sup> of each tier were sampled September 18, 1925. Water-soluble nitrates (NO<sub>3</sub>), total solids, volatile matter, and organic and inorganic CO<sub>2</sub> were determined on the aqueous extracts prepared according to the method of the Bureau of Soils.<sup>2</sup> The CO<sub>2</sub> was determined in the dry residue (total solids) obtained by evaporating in a suitable flask, aliquot of the clear water extract using the chromic acid method of White and Holben (10). The data secured from this study are shown in table 6. The burned lime plot 23 shows an excess of nitrates except on tier 2. In every instance with the exception of NO<sub>3</sub> on tier 2 the burned lime treated soil exceeds the check plot in water-soluble material. The burned lime plots show 36.8 per cent soluble organic matter in excess of the check plots. The two check plots on tiers 2 and 4, however, which have each received 3 tons of limestone show less soluble organic matter than the unlimed check plots of tiers 1 and 3.

#### EARLIER STUDIES OF BROWN AND MACINTIRE

In 1910 Brown and MacIntire (2) made a detailed study of water soluble nutrients of nine plots of tier 2. Included in the plots were those that have received burned lime with and without manure and the nearest check plot 24 and plot 16 treated with manure without lime. These studies included the determinations of nitrates (NO<sub>3</sub>), potassium, calcium, total solids, volatile matter, and total moisture. These determinations were made on samples taken at eighteen periods during the 1910 growing season. The data shown in table 7 have been recomputed from parts per million to a percentage relation where the results are expressed on the basis of the unlimed plots taken as 100. Thus if the unlimed plot shows 8.9 p.p.m. and the limed soil 9.2 p.p.m., the data are expressed as 103. This method of presentation, commonly used, avoids the necessity of including the actual unlimed data and aids in a rapid study of the relationship between the two treatments. A study of table 7 shows that as a general average there are no significant differences between the limed and unlimed plots with respect to the accumulation of nitrates. However, from May 6 to June 17 there appears a seasonal difference in the favor of the unlimed soil. For the remainder of the season the limed soils with a few exceptions exceed in nitrates. A study of the potassium data shows that there is no evidence that lime has increased the solubility of potassium compounds. The water-soluble calcium is greatly in excess on the limed plots. Lime used alone apparently slightly increased the water holding capacity, however, when

<sup>1</sup> From 1881 to 1921 no lime was used except on plots 22, 23, and 34. Beginning with 1922 all plots of tier 2 and 1923 tier 4 were treated with limestone except the plots previously limed and also two PK plots.

<sup>2</sup> U. S. Dept. Agr. Bur. Soils Bul. 31.

used with manure the limed plot shows the opposite effect. The volatile matter is in excess on both limed plots. On the basis of the data shown in table 6 the volatile matter on the CaO treated soils varies between 17 and 31

TABLE 7  
*Effect of lime on water soluble nutrients\**  
On the basis of the unlimed soils taken as 100

DATE SAMPLED	LIME (CaO) ALONE					LIME (CaO) AND MANURE				
	NO <sub>2</sub>	K	Ca	H <sub>2</sub> O	Volatile matter	NO <sub>2</sub>	K	Ca	H <sub>2</sub> O	Volatile matter
<i>1910</i>										
May 6.....	101	...	273	100	144	96	...	632	94	221
May 13.....	91	95	229	100	190	63	97	503	92	214
May 17.....	68	93	327	108	224	54	110	386	95	267
May 25.....	86	107	429	98	...	91	110	665	92	...
June 2.....	70	124	458	103	167	98	84	547	91	232
June 9.....	70	95	512	100	331	71	37	469	93	282
June 17.....	95	81	517	113	300	79	118	913	102	124
June 23.....	117	122	553	100	189	128	88	527	92	129
June 27.....	86	67	532	104	303	119	130	724	97	198
July 6.....	240	100	588	105	100	155	94	628	100	199
July 12.....	110	84	517	106	364	115	161	748	93	352
July 19.....	112	132	620	97	146	120	109	712	98	257
July 26.....	100	109	304	87	303	91	75	585	109	175
August 3.....	115	65	480	93	274	83	51	655	97	159
August 17.....	152	76	439	93	143	121	80	534	88	118
August 31.....	100	65	390	100	150	89	90	445	104	115
September 13....	88	75	437	105	280	72	136	486	98	170
September 30....	77	95	417	103	185	121	26	211	95	103

\* Studies made by Brown and MacIntre in tier 2, 1910. This tier was in oats in 1910.

TABLE 8  
*Volatile matter extracted by various organic solvents*  
On the basis of the unlimed soil taken as 100

	EACH SAMPLE SEPARATELY EXTRACTED		SUCCESSIVE EXTRACTIONS	
	Plot 22* (lime and manure)	Plot 23 (lime)	Plot 22 (lime and manure)	Plot 23 (lime)
Ether.....	61	108	45	90
Acetone.....	89	76	27	39
Chloroform.....	31	100	106	150
Ethyl acetate.....	64	77	92	108
Alcohol.....	60	76	103	103

\* Compared with plot 20 (10 tons manure) instead of plot 16 (6 tons manure).

per cent organic matter or an average of 23 per cent. The volatile matter on the two unlimed check plots shows 30 and 39 per cent organic matter compared

to 14 and 32 per cent on the limed check plots of tiers 2 and 4. Although there is necessarily a variation in the proportions of organic matter in the volatile material it is evident that the lime treatment has increased the water-soluble organic matter.

*Volatile matter ("organic matter") extracted by various organic solvents.* In 1909-1910 Brown, MacIntire, and Cree (3) conducted detailed studies of samples taken from tier 2 on October 11, 1909. Included in these studies were several experiments designed to determine the effect of lime on the solubility of "organic matter" in various organic solvents. The studies were carried out by means of the extraction apparatus devised by Wiley.<sup>3</sup> The data given

TABLE 9

*Analytical classification of nitrogen in decomposition products formed by digestion of soils and humus with acid\**

(In per cent of total nitrogen in soil and humus respectively)

PLOT NUMBER	PLOT TREATMENT	AM- MONIA-N	HUMIN NITROGEN			AMINO ACID NITROGEN	
			Soluble	Insolu- ble	Total	Mono- amino	Di- amino
By acid digestion of soil							
20	10 tons manure	21.64	28.32	26.29	54.61	8.63	12.97
22	6 tons manure and lime	18.39	25.36	28.02	53.38	17.48	9.04
23	Lime	16.32	31.77	29.09	60.86	12.80	8.21
24	Untreated	16.05	26.39	29.40	55.79	17.80	8.82
By acid digestion of humus							
20	10 tons manure	6.74	4.03	30.05	34.08	34.78	19.65
22	6 tons manure and lime	4.69	6.55	34.16	40.71	44.89	7.51
23	Lime	6.47	6.71	31.04	37.71	45.22	8.41
24	Untreated	10.63	5.69	19.65	25.34	55.02	7.29

\* Studies made by Brown and Lathrop.

in table 8 were obtained by extracting each soil for 7 hours. Two different methods of extraction were used. In one case each sample of soil was separately extracted, in the second case the same sample of soil was successively extracted in the order given.

The data in table 8 show that lime has reduced the solubility of volatile matter in the various solvents. The nature of the volatile matter was not determined; however, it is no doubt largely organic materials.

#### THE FORMS OF ORGANIC NITROGEN IN LIMED AND UNLIMED PLOTS

Brown and Lathrop (1) using soil samples taken in 1909 from a number of plots of tier 2 made a detailed study of the forms in which the organic nitrogen

\*Space will not permit a detailed account of the various experiments. The reader is therefore referred to the original papers.

occurs in the soil. The studies included: (a) treating the fine soil (0.5 mm.) with boiling acid, (b) treating humus prepared from these soils with boiling acid, and (c) heating the soils with water under pressure. The detailed methods used are included in the original article. Table 9 shows the results secured from a study of two limed and unlimed soils. A study of the results of Brown and Lathrop shown in table 9 brings out some interesting data concerning the effect of lime on the nitrogen compounds of the four soils. This is especially true in case of the soils of plots 23 and 24. There is a higher percentage of humin nitrogen on the limed plot 23, which according to the authors represents the unavailable portion of the total nitrogen. The mono-amino acid nitrogen is present in the unlimed soil in a much greater proportion. The data secured on plots 20 and 22 are not comparable since the two plots have received different proportions of manure. The humus studies show that the unlimed humus of plot 24 contains 62.31 per cent of its total nitrogen as amino

TABLE 10  
*Composition of alkali soluble humus*  
Per cent of ash free humus

	CARBON	HYDROGEN	OXYGEN	NITROGEN
Limed soil plot 23.....	52.12	5.80	38.709	3.371
Unlimed soil plot 24.....	41.20	6.10	49.893	2.806

acid nitrogen as compared to 53.63 per cent where lime was used. In other words, a larger proportion of the total humus nitrogen on the unlimed soil is potentially available for plant growth. A comparison of the availability of the humus nitrogen and total soil nitrogen shows that a much greater proportion of the humus nitrogen was converted into amino acid or available nitrogen material. From these figures we may conclude that the soil nitrogen associated with the alkali-soluble organic matter represents the more readily available organic nitrogen, and further that the effect of lime has been to reduce the availability of the humus nitrogen as measured by a larger proportion of humin nitrogen, and to reduce correspondingly the amino acid nitrogen.

#### COMPOSITION OF HUMUS FROM THE LIMED AND UNLIMED SOILS

Brown and Lathrop (1) included in their studies a detailed analysis of the humus derived from the limed and unlimed soils of plots 23 and 24 respectively. The original data are included in the same paper. Table 10 shows the results of this interesting study. The humus of the two soils shows a marked difference in composition. The humus of the limed soil shows a higher percentage of carbon and nitrogen.

## PHYSICAL EFFECTS OF LIME

A limited number of physical studies have been made on these old plots with reference to the effect of lime. The studies reported by Brown, MacIntire, and Cree (3) have been confined for the most part to the determinations of the

TABLE 11  
*Effect of lime on the behaviour of soil moisture\**  
Computed on basis of the unlimed soil taken as 100

	PLOT 23 (LIMED)	PLOT 24 (UNLIMED)
Apparent specific gravity.....	101	100
Water-holding capacity.....	96	100
Rate of downward movement of water:		
1 hour.....	437	100
5 days.....	227	100
11 days.....	207	100
Hygroscopic moisture.....	99	100
Water-absorbing capacity (7 days).....	106	100
Moisture in field soil:		
October 22.....	82	100
October 29.....	102	100
October 4.....	92	100
October 10.....	103	100
October 24.....	94	100
December 1.....	100	100

\* Studies of Brown and MacIntire.

TABLE 12  
*Effect of lime on soil structure as indicated by the draft of a plow*  
(On the basis of the unlimed soil taken as 100)

	HAY SOD			CORN STUBBLE			OATS STUBBLE		
	Tier 1 (1912)	Tier 2 (1913)	Tier 3 (1914)	Tier 4 (1912)	Tier 1 (1913)	Tier 2 (1914)	Tier 3 (1911)	Tier 4 (1912)	Tier 1 (1913)
Plot 23 (lime).....	90	98	102	100	97	100	100	100	92
Plot 24 (no lime).....	100	100	100	100	100	100	100	100	100
Plot 16 (manure).....	100	100	100	100	100	100	100	100	100
Plot 22 (lime and manure).....	99	97	99	98	97	107	91	100	93

effect of lime on the behaviour of soil moisture including: (a) water holding capacity, (b) hygroscopic moisture, (c) water absorbing capacity, and (d) rate of movement (drainage). Noll in 1912, 1913, and 1914 (6) determined the effect of the various manurial treatments on soil structure as measured by the plow draft. Tables 11 and 12 show a summary of these data. The results are



expressive on the percentage basis in which the unlimed soils are taken as 100. The moisture studies with the exception of the drainage data show no significant effect of lime on this soil. The rate of downward movement of soil water is shown to be over twice as rapid where lime has been applied.

The data in table 12 indicate that the draft of the plow has been somewhat reduced on plot 23 as compared to the unlimed soil of plot 24. On hay sod the average value is 96.7, on corn stubble, 99, and on oats stubble, 97.3. The lime used on plot 23 has apparently reduced the plow draft by 2.3 per cent as measured by a standard dynamometer. When lime was used with manure as compared to manure alone the average values are as follows: hay sod, 98.3, corn stubble, 100, and oats stubble, 94.7, equivalent to a general average of 97, or a reduction of the plow draft of 2.2 per cent. In only two out of eighteen trials did the lime soil show a heavier draft than the unlimed soil.

#### SUMMARY

The first three papers of the series dealing with a study of these old fertilizer plots have been confined largely to a study of the effect of lime on soil organic matter, including also certain studies made prior to 1921. It is the plan of the authors of this series to include when possible the results of the earlier studies which are now published in the annual reports of the College farm from 1899 to 1912. These earlier studies correlated with 1921 data are especially valuable in determining the progressive changes in these differently treated plot soils.

#### *Fate of burned lime and limestone (1881-1921)*

1. From 1881 to 1921 a total of 20 tons of burned lime were applied to plots 22 and 23 and 40 tons of limestone were applied to plot 34 (acre basis).

2. It is estimated on the basis of the chemical composition of the two lime materials that 35,200 pounds per acre CaO was applied as burned lime and 41,754 pounds CaO as limestone.

3. From 1881 to 1921, 64.4 per cent of the limestone used failed to pass a 0.25-mm. (100-mesh) screen. From 1881 to 1908, 79.34 per cent of the limestone applied was retained on a 0.25-mm. screen.

4. Examination of the limestone treated soil at the end of 40 years of continuous treatment showed that 17,631 pounds of carbonates had undergone decomposition.

5. The loss of carbonates was found to be confined to the fine material that originally passed a 0.25-mm. screen (of the 17,631 pounds of total  $\text{CaCO}_3$  decomposed, 17,434 pounds represented the very fine material).

6. Of the total CaO applied as burned lime 63 per cent of that used on the manured land was recovered in the surface soil as compared to 61 per cent where the lime was applied to the untreated soil.

7. It is estimated that of the 12,906 pounds CaO lost from the surface soil (0-7 inches) of plot 22 (lime and manure), 7715 pounds were lost mechanically

by being plowed down below the normal plowed depth, 836 pounds were removed in crops, and 4353 pounds were lost by drainage.

8. Where the burned lime was used without manure, of the 13,857 pounds CaO lost from the surface 7 inches, 9620 pounds were carried down mechanically by the plow, 502 were removed in crops, and 3735 pounds were lost by drainage.

9. On the land treated with limestone, only 13 per cent of the CaO was lost from the surface 7 inches. Of the 5407 pounds of CaO lost from the surface 7 inches, 3487 pounds were carried down below that depth by plowing, 550 pounds were removed in crops, and 1370 pounds were lost by drainage.

10. From the above figures it is shown that the actual loss of CaO from the normal plowed depth is not necessarily confined to that removed by drainage, in fact, only 33.8 per cent of the CaO lost from plot 22 is attributed to solution and subsequent drainage as compared to 27 per cent on plot 23 and 25.3 per cent on plot 34.

11. Of the total CaO found in the surface soil, 64 per cent on plot 22, 69 per cent on plot 23, and 82 per cent on plot 34 are present as  $\text{CaCO}_3$ .

12. The relatively low loss of lime from this soil during the forty years of continuous cropping may be due to several factors: (a) that the soil was no doubt alkaline at the beginning of the experiment, (b) that the soil is a heavy silt to clay loam not subject to excessive drainage, and (c) that during the non-growing season (October to March inclusive) the average air temperature at this station is  $34.3^\circ\text{F}$ . The soil therefore during the non-growing season is not subject to the same degree of bacterial activity as a soil farther south would be.

13. The relative decomposition of limestone on three differently treated soils is shown in table 4. The sulfate of ammonia treated soil decomposed the equivalent of 5954 pounds per acre  $\text{CaCO}_3$  in 30 days as compared to 2525 pounds on the untreated check plot soil and only 69 pounds on the soil treated with limestone for forty years. It is thus shown that the rate of limestone decomposition is influenced by the soil reaction that is, the relative supply of lime present.

#### *Chemical and physical effects of lime*

14. Hydrogen-ion studies show that the soil treated with burned lime has an average OH-ion concentration of 7.88 compared to 7.68 where limestone was applied.

15. Three months after burned lime was applied, the OH-ion concentration was 7.91 as compared to 7.81, 39 months after lime application. Lime has therefore not maintained a high OH-ion concentration on this soil.

16. The burned lime soil of tiers 1 and 3 contained as an average 63.7 per cent water-soluble organic matter and 66.4 per cent nitrates in excess of those on the unlimed check plot 24 of the same tiers.

#### *Results of earlier studies*

17. Studies made on these plots in 1910, including the determinations of water-soluble nutrients each week during the growing season, show that from

May 6 to June 27 the limed soil was deficient in nitrates as compared to the unlimed soil. During the remainder of the season the limed soil showed nitrates in excess of the unlimed soil.

18. Studies of water-soluble nutrients during seventeen periods of the growing season show that lime has had a tendency to reduce the water-soluble potassium by 7.1 per cent when used alone and 6.7 per cent when used with manure.

19. The studies of Brown and Lathrop show that lime has had the effect of increasing the proportion of humin or unavailable nitrogen in the soil and soil humus. The humus from the limed soil is also higher in percentage of carbon and nitrogen.

20. Studies of Brown, MacIntire, and Cree on the effect of the various treatments on the physical properties of the soil, show by laboratory experiments that lime apparently increases the rate of downward movement of soil water and reduces slightly the water-holding capacity of this soil. Moisture studies made in the field, however, show that there is no measurable effect of lime on the water-holding capacity of the same soils.

21. The field studies of Noll on the effect of the various treatments on the tilth or soil structure show that lime has somewhat reduced the plow draft. Lime used alone (plot 23) has reduced the plow draft 2.3 per cent and lime with manure (plot 22), 2.2 per cent.

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# CONCENTRATION OF CARBONATES IN TWO MINNESOTA SOIL TYPES<sup>1</sup>

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## INTRODUCTION

In the summer of 1923 when field work was commenced on a detailed soil survey of Lac qui Parle County, one of the Minnesota counties bordering South Dakota, the writer frequently observed in freshly made roadcuts a pronounced zone of carbonate accumulation, this being best developed on the well-drained upland. Lac qui Parle County lies in the glacial and loessial soil province of the United States in which the Barnes, confined to regions of low to moderate rainfall, is recognized as being one of the most prominent series. It has developed on calcareous glacial drift under grassland vegetation and has a dark brown to black surface soil with gray to brownish yellow, fine textured, highly calcareous subsoil. Its most outstanding characteristic, as recognized by the Bureau of Soils, is that when the soil has reached a mature development, there is a zone of carbonate accumulation at some horizon of the soil profile.

As no experimental data have been published showing the distribution or amount of carbonate in its profile an investigation was undertaken to determine how the carbonate content varied from the surface downward and especially how much more was present in the concentration zone than above and below.

The immediate surface of this soil is usually leached of carbonates and is slightly acid by the Truog test. Vigorous effervescence with dilute hydrochloric acid is usually encountered not more than about 16 or 18 inches below the surface, although in some places the subsoil to a depth of 30 inches will not effervesce. The carbonates in the subsoil may be uniformly distributed or may be collected in masses, often in well defined concretions.

As only a small part of the county had been surveyed at the time the samples used in this study were taken, no effort was made to confine the sampling to any particular soil types but 13 exposures were selected in which the carbonate accumulation was very evident. The soil survey of the county having since been completed, it has been found that two important types are

<sup>1</sup> Published with the approval of the Director as Paper no. 588 of the Journal Series of the Minnesota Agricultural Experiment Station.

TABLE 1  
*Effervescence and carbonate content of the soil profile sections*  
 The heavy lines indicate the upper and lower limits of the light gray color of the soils  
*Barnes silty clay loam*

DEPTH	PROFILE I		PROFILE II		PROFILE III		PROFILE IV		PROFILE V		PROFILE VI		PROFILE VII		AVERAGE
	Efferves- cence	Carbonate per cent	Efferves- cence	Carbon- ate	Efferves- cence	Carbon- ate	Efferves- cence	Carbon- ate	Efferves- cence	Carbon- ate	Efferves- cence	Carbon- ate	Efferves- cence	Carbon- ate	
<i>inches</i>															<i>per cent</i>
1-3	0	0.4	v. sli.	2.0	v. sli.	1.1	mod.	5.0	v. sli.	3.2	v. sli.	1.5	0	0.4	2.0
4-6	0	0.2	sli.	2.6	v. sli.	0.9	mod.	4.1	sli.	2.7	v. sli.	1.0	0	0.4	1.7
7-9	0	0.5	sli.	2.5	v. sli.	0.9	str.	9.8	sli.	3.0	v. sli.	1.4	0	0.4	2.7
10-12	0	0.4	str.	15.7	mod.	4.1	str.	16.8	str.	12.5	v. sli.	1.4	0	0.4	7.3
13-15	str.	26.6	str.	23.0	str.	17.3	str.	29.6	str.	17.7	sli.	2.3	0	0.3	16.8
16-18	str.	33.4	str.	30.0	str.	27.5	str.	35.2	str.	24.1	mod.	7.5	0	0.4	22.7
19-21	str.	23.9	str.	28.0	str.	30.7	str.	35.5	str.	25.7	str.	25.3	sli.	2.7	24.6
22-24	str.	25.5	str.	25.3	str.	26.6	str.	31.1	str.	25.0	str.	22.1	str.	20.0	25.1
25-27	str.	13.0	str.	23.9	str.	23.2	str.	24.3	str.	20.7	str.	23.4	str.	25.3	22.0
28-30	str.	14.1	str.	21.1	str.	20.2	str.	22.1	str.	24.3	str.	22.7	str.	27.1	21.7
31-33			str.	21.4	str.	16.8	str.	22.7	str.	22.1	str.	18.6	str.	27.3	21.5
34-36			str.	21.8	str.	17.3	str.	12.1	str.	17.3	str.	19.8	str.	29.6	19.7
37-39			str.	17.1	str.	17.1			str.	15.9	str.	13.4	str.	18.9	16.4
40-42			str.	15.9		15.9			str.	15.7	str.	22.3	str.	18.6	
43-45											str.	17.7			
Av. 1st foot....		0.4		5.7		1.7		8.9		5.4		1.3		0.4	
Av. 2nd foot....		27.4		26.6		25.5		32.9		23.1		14.3		5.9	
Av. 3rd foot....		13.6*		22.1		19.4		20.3		21.1		21.1		27.3	

\* Average of 2 sections.

0 = none; sli. = slight; mod. = moderate; str. = strong; v. = very.

## Unnamed sil loam

DEPTH	PROFILE VIII			PROFILE IX			PROFILE X			PROFILE XI			PROFILE XII			PROFILE XIII			AVERAGE
	Efferves- cence	Carbonate	per cent	Efferves- cence	Carbonate	per cent	Efferves- cence	Carbonate	per cent	Efferves- cence	Carbonate	per cent	Efferves- cence	Carbonate	per cent	Efferves- cence	Carbonate	per cent	
<i>inches</i>																			<i>per cent</i>
1-3	v. sli.	2.5		0	0.4	0	0	1.4	4.5	0	1.4	4.5	0	0.7	0.7	0	0.7	2.0	
4-6	v. sli.	2.5		0	0.2	0	0	2.0	3.4	0	2.0	3.4	v. sli.	2.5	2.5	v. sli.	2.5	2.1	
7-9	mod.	3.5		sli.	0.7	0	sli.	1.8	2.7	0	1.8	2.7	v. sli.	2.5	2.5	v. sli.	2.5	2.2	
10-12	str.	14.8		sli.	0.4	0	sli.	0.4	4.5	0	2.5	mod.	v. sli.	2.5	2.5	v. sli.	2.5	4.5	
13-15	str.	23.9		sli.	1.4	0	sli.	1.4	2.5	0	2.5	v. sli.	0	2.7	2.5	0	2.7	8.7	
16-18	str.	27.3		mod.	2.7	0	mod.	2.0	2.0	0	2.0	v. sli.	v. sli.	2.7	2.5	v. sli.	2.5	11.3	
19-21	str.	29.1		str.	21.6	0	str.	2.0	4.5	0	2.0	sli.	4.5	3.0	3.0	v. sli.	3.0	15.0	
22-24	str.	25.3		str.	30.5	mod.	str.	4.6	21.1	mod.	4.6	str.	21.1	2.7	2.7	v. sli.	2.7	18.1	
25-27	str.	18.4		str.	30.7	str.	str.	34.3	24.0	str.	34.3	str.	24.0	10.2	10.2	str.	10.2	23.0	
28-30	str.	17.8		str.	30.5	str.	str.	45.5	32.1	str.	45.5	str.	32.1	25.9	25.9	str.	25.9	28.9	
31-33	str.	17.5		str.	29.6	str.	str.	39.1	36.4	str.	39.1	str.	36.4	31.1	31.1	str.	31.1	29.6	
34-36	str.	16.6		str.	20.9	str.	str.	35.7	27.1	str.	35.7	str.	27.1	30.5	30.5	str.	30.5	26.0	
37-39				str.	25.3			30.9	21.6	str.	30.9	str.	21.6	26.6	26.6	str.	26.6		
40-42				str.	28.9			26.8	21.5	str.	26.8	str.	21.5	21.6	21.6	str.	21.6		
43-45				str.	30.0			22.7		str.	22.7	str.							
46-48				mod.	10.0			20.9		str.	20.9	str.							
49-51				mod.	7.7					str.									
Av. 1st foot.....		5.8			0.4			1.9	3.8		1.9			2.1	2.1		2.1		
Av. 2nd. foot....		26.4			14.0			2.8	7.7		2.8			2.7	2.7		2.7		
Av. 3rd. foot.....		17.6			27.9			38.6	29.9		38.6			24.4	24.4		24.4		

0 = none; sli. = slight; mod. = moderate; str. = strong; v. = very.

represented in the samples collected, one the Barnes silty clay loam, the other a silt loam of apparently loessial origin, but unnamed, as final correlation has not yet been made by the United States Bureau of Soils. This type, as found in Lac qui Parle County, may be described as follows:

The surface soil to a depth of 8 inches consists of a black to very dark brown silt loam which has a single grain or silty structure and is loose and friable. This is underlain to a depth of 14 inches by a dark brown horizon somewhat finer in texture and more compact than the surface layer. The next, or third horizon, which extends to a depth of 24 inches, has a yellowish brown color distinctly lighter than the horizon immediately overlying it. Its texture is finer, being a silty clay loam or silty clay, and the material comprising it is compact, somewhat tough, and quite plastic when wet. These three upper horizons have been leached of their carbonates. The fourth, the zone of carbonate concentration, begins abruptly below the heavy horizon and extends to a depth of 34 inches, having a thickness of from 4 to 8 inches. It consists of loose, friable silty material ranging in color from light yellowish gray to almost white and contains lime concretions. Below this layer is a friable, yellowish-brown silty clay loam which is uniformly calcareous and has no concretions of lime.

Six of the sets taken are from this type and seven from the Barnes.

#### METHOD OF SAMPLING

Samples were taken only at places where a careful preliminary examination showed a distinct zone of carbonate accumulation. As it is certain that not all the mature soils of these series have zones with so much carbonate, the locations selected may represent those of maximum accumulation, and areas of these types may be found with little or no accumulation. When a satisfactory profile had been found, a fresh vertical face was exposed, by cutting back into the bank with a spade, and this smoothed and then marked off into 3-inch sections to a depth that in most cases extended below the distinct zone of carbonate accumulation. Commencing with the lowest marked section, in order to avoid any contamination from the overlying soil, and working upward, using a small garden trowel, samples were taken every 3 inches. About 3 pounds of soil was removed from each section, placed in a pail, and thoroughly mixed. A sample of this was removed, placed in a sack, and shipped to the laboratory. After being allowed to become thoroughly air dry it was passed through a 2-mm. sieve, any portion remaining on this being removed and pulverized in a porcelain mortar, a rubber tipped pestle being used. All pebbles and stones were discarded. Frequently small concretions of calcium carbonate, more than 2 mm. in diameter, composed a part of the sample. These were not discarded with the pebbles, but, being considered part of the carbonate concentration, were crushed and included in the fine material used for the CO<sub>2</sub> determination.

All the samples were taken the latter part of September, 1923.

#### METHOD OF CARBONATE DETERMINATION

In order to determine the carbon dioxide content, a small weighed quantity of the finely divided sample was placed in a small flask and treated with a

measured volume of standard 0.1 *N* hydrochloric acid solution. After vigorous evolution of gas had ceased, the vessel was gently warmed over a flame until all reaction had stopped. The excess, or unneutralized acid, was determined by titrating with a 0.1 *N* solution of sodium hydroxide and the percentage of carbonate calculated. As all the carbonate is assumed to have been present as calcium carbonate, the computed percentages of carbonate are reported on this basis.

Table 1 gives the percentage of carbonate and the effervescence for each 3-inch section of each profile for both soil types. Figures 1 and 2 show the vertical distribution of carbonate by 3-inch sections.

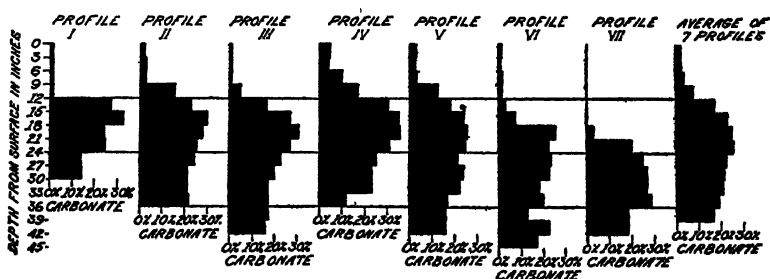


FIG. 1. DISTRIBUTION OF CARBONATE IN BARNES SILTY CLAY LOAM

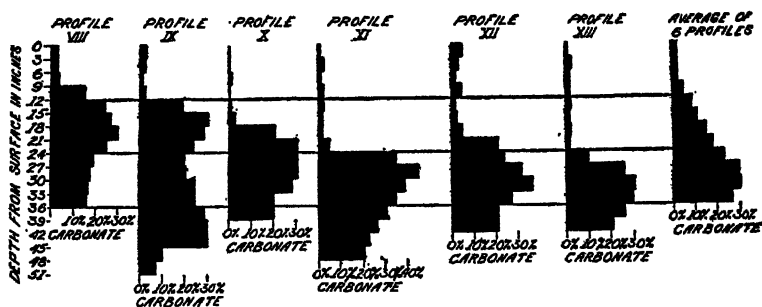


FIG. 2. DISTRIBUTION OF CARBONATE IN UNNAMED SILT LOAM

#### DISCUSSION

It will be seen from figures 1 and 2, that in every profile there is a pronounced zone of carbonate accumulation, occurring relatively close to the surface in some cases and deeper in others. In a soil derived from parent glacial material of high carbonate content it is difficult to decide definitely, in the case of some of the profiles, at just what depth the carbonate accumulation commences, and even more difficult to decide where it ends. This is particularly true of profiles IV, V, and VIII. With profile IV the carbonate content begins to increase 6 inches below the surface and for each additional



3-inch section it increases until it reaches its maximum at a depth of 18 inches, from which point it gradually diminishes until at 34 inches it is about the same as at 9 inches. The maximum accumulation in the Barnes silty clay loam is found at an average depth of 21 inches, whereas in the unnamed silt loam it occurs at a slightly greater depth—about 30 inches. In some of the profiles considerable carbonate is present even in the immediate surface layers, as 3.2, 5.0 and 4.5 per cent in the first 3-inch sections of profiles V, IV, and XII. Its presence here, however, does not seem to influence the depth at which the concentration zone occurs nor does it, when compared with the other profiles, appear to increase or diminish the total amount present in that zone. The profile showing the most pronounced accumulation is that of XI (fig. 2), in the 21–24-inch section of which there is only 4.6 per cent of carbonate, whereas in the section immediately below there is 34.3 per cent, reaching its maximum of 45.5 per cent in the next 3-inch section, and from this gradually dropping until at a depth of 48 inches there is only 20.9 per cent. In practically all cases as the zone is approached from the surface there is a very abrupt rise in the carbonate content but as the point of maximum accumulation is passed the drop is more or less gradual. Exceptions to this, however, are noted in profiles IX and XII where there is a more pronounced break. An interesting feature is to be observed in profile IX (fig. 2) in which it will be seen that two concentration zones occur, the upper one beginning at 15 inches below the surface and the lower one at a depth of 40 inches. Both these zones were plainly visible, having concretions of carbonate embedded throughout the soil mass.

For both of the soil types the average content of carbonate in the successive 3-inch sections was computed, this being shown at the extreme right in figures 1 and 2. From these it will be seen that the irregularities, so pronounced in each individual profile, have been largely eliminated and in each there is a more gradual rise and fall of the carbonate, but with the zone of accumulation standing out prominently with each type.

In this connection it is of interest to note the very large amount of carbonate present in these two types. At the point of maximum accumulation the carbonate content ranges from 25.3 to 45.5 per cent and in the substratum of parent material, below the zone of accumulation, the carbonate content ranges from 7.7 to 22.7 per cent.

#### RELATION OF CARBONATE CONTENT TO TEXTURE

Five of the sets showing pronounced concentration zones were subjected to moisture-equivalent determinations in order to see whether any relation existed between the carbonate content and the texture. As this zone in all cases lies below the surface soil and well away from the influence of the large proportion of organic matter which characterizes the surface of these types and greatly affects the moisture equivalent, the values may be assumed to give a single-valued expression of texture and therefore to be directly

comparable one with another. If the high content of carbonate in some of the sections were instrumental in either decreasing or increasing the moisture equivalent this would have been brought out. In table 2 are shown the percentages of carbonates in these profiles and the moisture equivalents. Apparently no direct relation exists between the two values, the texture remaining practically constant both above and below the zone of carbonate accumulation. However, in the case of one profile, no. I, the moisture equivalent is highest at the point of maximum accumulation and falls off rather sharply below this.

TABLE 2  
*Moisture equivalents and calcium carbonate for 5 soil profiles*

DEPTH	BARNES SILTY CLAY LOAM						UNNAMED SILT LOAM			
	Profile I		Profile III		Profile IV		Profile X		Profile XII	
	Moisture equivalent	Calcium carbonate	Moisture equivalent	Calcium carbonate	Moisture equivalent	Calcium carbonate	Moisture equivalent	Calcium carbonate	Moisture equivalent	Calcium carbonate
		per cent		per cent		per cent		per cent		per cent
<i>inches</i>										
1-3	26.8	0.4	36.1	1.1	33.3	5.0	32.8	0.4	29.9	4.5
4-6	24.3	0.2	31.2	0.9	30.6	4.1	32.9	0.2	30.3	3.4
7-9	22.4	0.5	28.6	0.9	32.3	9.8	28.1	0.7	30.0	2.7
10-12	22.9	0.4	26.6	4.1	31.5	16.8	27.7	0.4	28.3	4.5
13-5	27.1	26.6	25.3	17.3	33.9	29.6	26.9	1.4	25.9	2.5
16-18	32.7	33.4	27.7	27.5	34.8	35.2	27.7	2.7	25.9	2.7
19-21	26.7	23.9	28.8	30.7	34.3	35.5	27.2	21.6	23.7	4.5
22-24	12.4	25.5	27.5	26.6	31.5	31.1	28.5	30.5	21.4	21.1
25-27	8.9	13.0	26.7	23.2	28.7	24.3	29.7	30.7	21.3	24.0
28-30	9.1	14.1	26.1	20.2	25.3	22.1	30.1	30.5	23.9	32.1
31-33			26.4	16.8	18.5	22.7	31.5	29.6	27.1	36.4
34-36			27.3	17.3	19.6	12.1	28.8	20.9	25.2	27.1
37-39			26.9	17.1					23.8	21.6
40-42			26.2	15.9						21.5

#### RELATION OF CARBONATE CONTENT TO COLOR

Because of the high percentage of carbonate in the concentration zone and its pronounced whitish color, as observed in the field at the time of sampling, color comparisons of the samples collected were made in order to determine whether any close relation existed between the carbonate content and the color. For this purpose small samples from each section of every profile were placed on trays and arranged in order of depth. Four color groups, consisting of (a) black to very dark brown, (b) grayish brown, (c) light gray to light yellowish gray and (d) yellowish gray, were chosen as being those into which all the soils could be grouped. The surface layers of all profiles, ranging in depth from 9 to 32 inches, were dark and therefore were placed in groups a and b. As depth increased, the change from dark to light was, in nearly all profiles, abrupt, the color becoming light gray to light yellowish

gray, and as this was passed the soil became a distinct yellowish gray. The light gray to light yellowish gray color of the soils is indicated in table 1 by heavy, black lines placed at the points where the gray color was first observed and where it ended. It will be seen that these lines inclose the sections comprising the concentration zones and agree very closely with these as shown in figures 1 and 2.

In the case of profile IX which has the double zone, it will be seen that the 22-33 sections, although containing a relatively high percentage of carbonate, are not enclosed in the lines; the soils of these sections were distinctly yellow in color.

#### DEGREE OF EFFERVESCENCE

The degree to which the soils effervesced when treated with hydrochloric acid was determined for all 3-inch sections of each profile. Approximately 5 gm. of air-dry soil was placed on a watch glass and about 10 cc. of dilute hydrochloric acid added. The degree to which the soils effervesced is indicated in table 1 as slight, moderate, and strong. It will be seen that there is a very close agreement between the degree of effervescence of each section of all profiles and the carbonate content. In the case of each profile there was very strong effervescence in the sections of maximum carbonate content although there also was strong effervescence in the layers above and below the zone if the carbonate content exceeded 10 per cent.

If five degrees of effervescence be recognized, the following values show the maxima, minima, and average:

DEGREE OF EFFERVESCENCE	NUMBER OF SAMPLES SHOW- ING THIS	CARBONATE CONTENT		
		Maxima	Minima	Average
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
None.....	21	2.7	0.2	1.0
Very slight.....	23	3.2	0.9	2.1
Slight.....	13	4.5	0.4	2.6
Moderate.....	9	10.0	2.7	5.5
Strong.....	110	45.5	9.8	23.7

#### SUMMARY

Two important soil types in western Minnesota, at depths varying from 12 to 27 inches below the surface, show zones of pronounced carbonate accumulation, in which lime concretions are thickly distributed and in which the carbonate content ranges from 25.3 to 45.5 per cent. In the unaltered material below, it varies from 7.7 to 22.7 per cent.

No direct relation was found between the carbonate content and the fineness of texture of the subsoil.

In the sections of carbonate concentration the color was distinctly grayer than in those above and below.

# THE COMPOSITION OF BIENNIAL WHITE SWEET CLOVER AS RELATED TO SOIL ENRICHMENT

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The increasing use of the various sweet clovers, especially the biennial white variety, for soil enrichment and for other purposes, all of which are eventually related to soil fertility, suggested the need for more information on the chemical composition of this crop. The purpose of this study was to determine the composition of the tops and of the roots at various stages of growth, in order more accurately to advise how to handle this clover in the various cropping systems.

The literature contained no systematic studies dealing with the composition of sweet clover, with the exception of some nitrogen analyses. Since the completion of this work, Willard (4) has published data from field experiments on the nitrogen content and yield of tops, stubble, and roots of the biennial white sweet clover, when seeded with oats.

The samples analyzed in the work reported here were collected from a limited area seeded for the purpose of this study. The soil type on which the samples grew was a brown silt loam, the surface of which was slightly acid, and the subsurface and subsoil of which contained limestone particles. Successive adjacent samples were taken. Every precaution was taken to obtain uniformly developed plants. The root samples were taken 14 inches deep. The tops were separated from the roots at the time of collection. Duplicate analyses were made on all samples. The chemical methods employed were the recognized methods for plants: the total nitrogen method included nitrates; and sulfur was determined by the bomb method. In most cases about thirty plants constituted the sample, although the one of October 15 contained one hundred plants. All figures are reported on the water-free basis. The results are given in pounds per ton and on a percentage basis in separate tables, for convenience in comparison with other similar data.

## COMPOSITION OF TOPS AND ROOTS, GROWTH OF FIRST SEASON

The composition of the fall growth of tops and roots, which was determined first, is presented in tables 1 and 2. The complete data appear in table 5.

<sup>1</sup>This work was conducted while the authors were on the staff of the Agronomy Department of the University of Illinois. The senior author is now connected with the University of Wisconsin, and the junior author is on the Ohio State University staff.

The tops decrease in nitrogen, phosphorus, sulfur, and potassium, but change very little in calcium and magnesium content. One sample is out of line with

TABLE 1  
*Changes in composition of sweet clover tops (first season's growth)*  
Pounds per ton on water-free basis

DATE	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1919</i>						
July 25.....	62.40	4.88	12.48	31.46	30.36	11.08
September 10.....	60.80	3.10	10.56	25.82	30.56	9.56
October 10.....	55.80	2.26	10.28	19.46	32.66	11.34
October 15.....	47.00	2.22	10.10	21.82	28.36	9.94
October 25.....	43.00	2.16	8.90	15.88	29.76	10.00
November 1.....	56.00	5.94	8.10	32.74	38.58	7.66
December 2.....	30.00	0.92	5.56	6.64	18.14	6.80

TABLE 2  
*Changes in composition of sweet clover roots (first season's growth)*  
Pounds per ton on water-free basis

DATE	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1919</i>						
October 15.....	61.60	5.00	4.58	12.14	6.14	5.66
October 25.....	59.80	5.84	5.06	14.40	6.26	5.18
November 1.....	71.40	8.92	3.84	22.16	6.54	4.48
December 31.....	66.20	4.80	5.48	15.26	5.86	3.74

TABLE 3  
*Changes in composition of sweet clover tops (second season's growth)*  
Pounds per ton on water-free basis

DATE	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1920</i>						
May 10.....	82	5.64	10.24	27.74	31.10	13.58
May 20.....	62	3.95	9.44	26.68	26.04	10.04
June 3.....	49	3.56	8.46	37.54	23.64	9.46
June 19.....	33.4	2.50	7.82	23.94	14.56	8.64
July 3.....	34.2	2.46	6.86	16.46	17.32	9.22
July 17.....	36	2.30	6.20	15.60	17.08	8.04
August 1.....	36	2.16	5.70	13.92	18.96	7.46
August 14.....	24.4	1.48	5.34	12.74	14.82	5.40
August 28.....	26	1.16	4.00	20.04	11.60	4.66
September 11.....	27.2	1.28	3.98	12.96	7.80	3.96
October 1.....	26	1.50	3.58	11.70	9.46	4.36

the others for some unknown reason, although special attention was given this sample to determine the cause of its higher content of nitrogen, phosphorus,

potassium, and calcium. No evidence of irregular soil condition could be obtained, and the only tenable assumption seemed to be that it had renewed its growth of tops because of favorable weather conditions. The high calcium and phosphorus content suggested the presence of a phosphate in the area of the soils from which this sample was taken. Similar difficulties in composition studies in successive sampling have been reported by other workers.

The loss in nitrogen, phosphorus, sulfur, and potassium in the tops is gradual and more regular than would be accounted for by the action of rain. As care was exercised to avoid loss of leaves no change in composition can be attributed to that possibility. This decrease was not accompanied by an increase in dry matter, except on the earliest dates. The calcium and magnesium decreased very slightly and then only at the last sampling, disregarding the

TABLE 4  
*Changes in composition in sweet clover roots (second season's growth)*  
Pounds per ton on water-free basis

DATE	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
<i>1920</i>						
March 20.....	70.2	5.06	6.00	11.00	6.00	5.54
April 24.....	73.8	5.16	8.26	10.82	10.32	8.02
May 10.....	71.4	3.66	8.50	11.50	9.32	7.24
May 20.....	57.4	2.30	7.74	11.12	9.74	7.54
June 3.....	43.6	2.28	8.64	11.18	9.32	7.86
June 19.....	40.0	2.20	7.66	19.94	8.66	8.56
July 3.....	33.0	1.92	6.04	12.68	8.54	8.40
July 17.....	24.0	1.80	6.10	16.42	9.00	8.40
August 1.....	27.4	1.66	5.94	19.82	9.40	8.38
August 14.....	27.2	1.46	5.86	19.08	10.66	8.34
August 28.....	21.6	1.12	5.76	31.48	11.76	6.98
September 11.....	15.6	1.36	4.06	21.72	8.54	7.06
October 1.....	18.4	1.90	3.86	21.00	10.66	6.32

November 1 sample. One would predict that if a crop is to store elements for the next season's growth, nitrogen, phosphorus, and sulfur would be the elements most concerned in the process.

An examination of the root composition shows larger amounts of nitrogen and phosphorus than those found in the tops. It is also noted that the roots gained in these elements at the same time that they increased in weight of dry matter. The roots are much richer in nitrogen and phosphorus than the tops of the same plants on the same dates. This is not true of the other elements. From this fact, one may assume that the plant stores nitrogen and phosphorus, but does not translocate all the mineral elements, except in a very limited way. The data do not prove a translocation of the material from the tops to the roots, but suggest it very strongly for nitrogen and phosphorus and slightly for sulfur. The roots are richest in nitrogen and potassium,

followed by calcium and then by phosphorus, sulfur, and magnesium; among the last three there is little difference.

TABLE 5  
*Composition of sweet clover tops and roots*  
Percentages on water-free basis

DATE	PART OF PLANT	PROPOR- TION OF WHOLE PLANT	NITROGEN	PHOS- PHORUS	SULFUR	POTAS- SIUM	CALCIUM	MAGNE- SIUM
<i>1919</i>		<i>per cent</i>						
July 25.....	Tops		3.12	0.244	0.624	1.573	1.518	0.554
September 10.....	Tops		3.03	0.155	0.528	1.291	1.528	0.478
October 10.....	Tops		2.79	0.113	0.514	0.973	1.633	0.567
October 15.....	Tops	42	2.35	0.111	0.505	1.091	1.418	0.497
October 15.....	Roots	58	3.08	0.250	0.229	0.607	0.307	0.283
October 25.....	Tops	37	2.15	0.108	0.445	0.794	1.488	0.500
October 25.....	Roots	63	2.99	0.292	0.253	0.720	0.313	0.259
November 1.....	Tops	..	2.80	0.297	0.405	1.637	1.929	0.383
November 1.....	Roots	..	3.57	0.446	0.192	1.108	0.327	0.224
December 2.....	Tops	..	1.50	0.046	0.228	0.332	0.907	0.340
December 31.....	Roots	..	3.31	0.240	0.274	0.763	0.293	0.187
<i>1920</i>								
March 20.....	Roots		3.51	0.253	0.300	0.550	0.300	0.277
April 24.....	Roots		3.69	0.258	0.413	0.541	0.516	0.401
May 10.....	Tops	45	4.10	0.282	0.512	1.387	1.555	0.679
May 10.....	Roots	55	3.57	0.183	0.425	0.575	0.466	0.362
May 20.....	Tops	48	3.10	0.197	0.472	1.334	1.302	0.502
May 20.....	Roots	52	2.87	0.115	0.387	0.556	0.487	0.377
June 3.....	Tops	61	2.45	0.178	0.423	1.877	1.182	0.473
June 3.....	Roots	39	2.18	0.114	0.432	0.559	0.466	0.393
June 19.....	Tops	80	1.67	0.125	0.391	1.197	0.728	0.432
June 19.....	Roots	20	2.00	0.110	0.383	0.997	0.433	0.428
July 3.....	Tops	86	1.71	0.123	0.343	0.823	0.866	0.461
July 3.....	Roots	14	1.65	0.096	0.302	0.634	0.427	0.420
July 17.....	Tops	82	1.80	0.115	0.310	0.780	0.854	0.402
July 17.....	Roots	18	1.20	0.090	0.305	0.821	0.450	0.420
August 1.....	Tops	78	1.80	0.108	0.285	0.646	0.948	0.373
August 1.....	Roots	22	1.37	0.083	0.297	0.991	0.470	0.419
August 14.....	Tops	70	1.22	0.074	0.267	0.637	0.741	0.270
August 14.....	Roots	30	1.36	0.073	0.293	0.954	0.533	0.417
August 28.....	Tops	73	1.30	0.058	0.200	1.002	0.580	0.233
August 28.....	Roots	27	1.08	0.056	0.288	1.574	0.588	0.349
September 11.....	Tops	76	1.36	0.064	0.199	0.648	0.390	0.198
September 11.....	Roots	24	0.78	0.068	0.203	1.086	0.427	0.353
October 1.....	Tops	79	1.30	0.075	0.129	0.585	0.473	0.218
October 1.....	Roots	21	0.92	0.095	0.193	1.050	0.533	0.316

When the relative amounts of the elements in the roots and tops are compared, the importance of nitrogen and phosphorus for maintaining the plant through the winter is evident.

The importance of sulfur in the plant metabolism is suggested by the large amounts—three to four times greater than the phosphorus—contained in the tops. This will be taken up later in the discussion.

There is not sufficient evidence in these figures to warrant the statement that the roots are absorbing plant-food elements from the soil after the top growth has ceased, but this is a possible explanation of some of the variations in root composition.

#### RELATION OF ROOT AND TOP COMPOSITION TO FALL PLOWING, GROWTH OF FIRST SEASON

Fall plowing is almost a necessity in some sections and is desirable on many farms.

The composition of the roots of sweet clover of the first year is less in percentage and in total amount of plant-food than in the spring of the second year. The weight of organic matter is much less the first year. It is thus evident that soils needing enrichment in nitrogen and organic matter will be helped more by spring plowing than by fall plowing of biennial white sweet clover. The need for improvement of the surface and subsurface should determine the time of fall plowing, if a crop is not to be seeded in the fall after plowing. Plowing early in the fall incorporates in the soil, young tender tops and roots, both of which proceed to decompose rapidly. Such early plowing results in conditions which will cause relatively large losses during the fall and spring. A much larger part of the crop is incorporated in the surface soil when plowed early. Early fall plowing is more satisfactory for killing the crop than late fall plowing. If a crop is to follow immediately, early fall plowing would be justifiable, and would result in considerable enrichment of the surface soil if systematically followed, as can be seen by reference to the analyses of the early fall tops.

Late fall plowing would offer a better opportunity for enriching the deeper layers of the soil, because of the roots having developed more fully and being richer than at earlier stages. The surface is also improved at the same time, as it must be remembered that at all times the largest part of the roots and tops will be plowed into the surface 7 or 8 inches. The improving of the sub-soil will be a much slower process than the improving of the surface or sub-surface. Both are equally important, and both should be enriched at the same time, if possible, although the slower rate of enriching the lower layer means that it must receive consideration for a longer time. At any given period the weight of roots below 14 inches is a small proportion of the total root weight. The root development at deeper layers depends upon the acidity and permeability of the soil type. An increasing proportional root development may occur at lower layers through repeated growth of deep-rooted crops that are plowed at a time when rich, plump roots are present in those layers. This will insure leaving the elements of fertility at the deeper layers and in the most decomposable forms, regardless of where they originated.



The rate of nitrification of the late fall growth of sweet clover is less than of the early growth, especially of the tops. This would help check the losses from the practice. Fall plowing, as commonly practiced, is not successful in killing the crop. Cutting a hay crop before translocation, and following it with plowing before growth proceeds much, should aid in killing the fall growth. This effect will depend, however, upon weather conditions prevailing thereafter, and upon how much the roots may have stored before cutting occurred. Cutting the hay crop early and delaying plowing enables the crop to recover its loss of food, and killing is not then effectively accomplished. The vigor of this biennial weed has not yet been reduced by cultivation, and its survival under even adverse conditions is common. The composition of fall cut hay may be judged from the analyses.

TABLE 6  
*Composition of sweet clover, leaves, stems, roots and seeds*  
Pounds per ton on water-free basis

DATE	PART OF PLANT	PROPORTION OF WHOLE PLANT	NITROGEN	PHOSPHORUS	SULFUR	POTASSIUM	CALCIUM	MAGNESIUM
		<i>per cent</i>						
<i>1919</i>								
October 15.....	Leaves	16	68.40	3.61	15.84	22.40	57.68	10.74
October 15.....	Stems	24	27.00	1.44	4.24	18.20	14.68	2.18
October 15.....	Roots	60	61.20	4.04	4.82	12.80	6.54	4.84
<i>1920</i>								
July 3.....	Leaves	20	70.80	4.00	15.66	22.40	44.06	10.04
July 3.....	Stems	66	22.80	1.99	3.98	14.80	8.94	1.84
July 3.....	Roots	14	32.00	1.92	6.04	12.60	8.54	8.40
August 28.....	Seeds		99.00	8.99	9.46	16.00	21.62	2.96
September 11.....	Seeds		94.40	8.39	8.80	14.00	22.42	2.78

#### DISTRIBUTION OF THE ELEMENTS IN THE FIRST SEASON'S GROWTH

Nitrogen is contained in both the tops and roots in larger amount than the other elements reported. In a sample of the root nitrogen studied, 86 per cent was water-soluble after grinding. In the stem and leaves only about one-third of that present is soluble. The high solubility of nitrogen in the root accounts for the rapid rate of decomposition of the roots in spite of their woody appearance. This plant stores its nitrogen in a soluble form that is easily moved even under cool growing conditions. This nitrogen is known to be largely organic and is apparently composed of the simpler building stones of legume proteins.

Calcium was found to occupy first place among the mineral elements in the fall tops and second place among them in the fall roots. In fact, in the whole fall plant it exceeds potassium slightly, being second to nitrogen. This high

calcium content is in accord with its large dependence upon the presence of limestone in the soil. There is, however, another significant fact shown by the analyses of the leaves in table 6. Calcium is present in the leaves in very large amounts. Its presence in such amounts in the plant's laboratory suggests it has, among others, a special function in this plant in the manufacture of the nitrogen compounds. It apparently becomes fixed in rather stable forms, otherwise it would be moved about and lost by leaching. It even considerably surpasses potassium in the sweet clover seed. Ames and Boltz (1) reported that the calcium of alfalfa was the least soluble of the three mineral elements, being less than half as soluble as potassium and magnesium, and that it existed in some organic form other than as the oxalate.

Potassium is the third element in quantity in the tops, and second in the roots. The response of sweet clover to potassium feeding is shown by its high content of that element where it has been applied.

Magnesium is present in the tops in much smaller amounts than calcium and potassium. The magnesium content of the seed is very low, which coincides with similar analyses of alfalfa seed, but is directly opposite to that of common clovers. The magnesium content of the leaves of the fall growth greatly exceeds that of the seed, which in turn exceeds that of the stems.

Sulfur is present in the tops in larger amounts in most cases, and in the roots in lesser amounts than the phosphorus. Sweet clover is a sulfur feeder, as judged by these results. It means that large amounts may be removed in the hay. Its high content of sulfur may be attributed to its preventing this element from leaching out of the soil by absorbing it during periods when it would otherwise be lost. At the Spring Valley Experiment Field some sulfur is obtained from the fumes of the heating plant adjacent to it. Three top and root samples taken for the authors by Snider and Hein of the soil experiment field staff on September 11, 20, and 27, 1922, showed 13.72, 14.00 and 14.40 pounds, respectively, of sulfur per ton of tops; and 9.40, 7.20, and 8.20 pounds respectively for the corresponding roots. Whether these large quantities are in excess of the optimum requirements is not known. Whatever the case, this high content is important in considering the sulfur cycle.

On twelve plots studied Ames and Boltz found the average sulfur content of alfalfa hay of first and second cutting higher than that of phosphorus. In alfalfa the sulfur was only 50 per cent soluble, which suggests its close relation to the nitrogen metabolism in such legumes.

Phosphorus is contained in sweet clover in the smallest amount of all the important elements. This deserves special consideration in using the crop for soil enrichment and will be treated under another heading.

#### COMPOSITION OF TOPS AND ROOTS OF GROWTH OF SECOND SEASON

The roots in the spring of the second year appeared to increase in all the elements, except potassium, before a top growth developed. This is shown in table 5. There is not sufficient data to decide whether the increase is real or

apparent. An extension of the root system in cold weather, and an early functioning of new and old nodules probably accounts for the increases noted. The tops of April 24 were about two inches high. Between April 24 and May 10, rapid changes occurred because of a rapid growth of tops.

A gradual decrease in percentage or pounds per ton is seen in the tops for nitrogen, phosphorus, sulfur, calcium, and magnesium, and a general decrease, with two exceptions, for potassium. These decreases were accompanied by large increases in dry matter, as the plants approached maturity.

In the roots nitrogen, phosphorus, and sulfur decrease in an orderly manner, as in the tops. This is shown in table 4. The calcium and magnesium tend to change very little, compared with the other elements. Potassium is again the exception, being very high on several dates and varying from 11 to 31 pounds. In fact, it tends to increase instead of decrease. The reasons for the large potassium content of the tops on June 3 and August 28 and in the roots on June 19 and August 28, are not known, but the authors suggest that this element may be needed in large amounts at special periods in the plant's development, such as preceding flowering and during seed ripening. These large amounts may represent a tolerance, but this seems unlikely, as the other elements are not present in large amounts on those dates.

#### RELATION OF ROOT AND TOP COMPOSITION TO SPRING PLOWING, GROWTH OF SECOND YEAR

Nitrogen is the most important element in the sweet clover from the standpoint of its use in improving soils and supplying nitrate rapidly for the succeeding crop. The amount of nitrogen in the whole plant in the spring depends upon the weather conditions that prevail. In some seasons the crop was found to contain about 200 pounds of nitrogen per acre in the tops alone by May 10. In other seasons the tops contained about 75 pounds, because of a growth retarded by the season being later. Soils needing enrichment in nitrogen and organic matter should have as much of the second year growth plowed under as possible without danger of injury to the succeeding crop.

After the crop has been successful on the same field two or three times, it may be plowed under earlier, because of less need for the largest amount of active organic matter.

The enrichment of the subsurface and subsoil will be accomplished more rapidly if the crop is plowed after the roots have started to increase in mineral elements and nitrogen in the spring, but before much material has moved into the tops from the roots. The results show this date to be about May 10 for the conditions thus far studied.

If no crop is to follow the sweet clover, it may well be left to complete its full growth and to add all the material to the soil. When so left to mature, the rate of nitrification is very slow if the mature dried material is plowed under in the fall. This is a decided advantage, as less nitrate is lost under these conditions.

## COMPOSITION OF LEAVES, STEMS, ROOTS, AND SEEDS

The composition of the leaves, stems, and roots, in fall and summer samples, and also the analysis of samples of seeds are included in table 6.

The richness of the leaves and the importance of conserving them is apparent from the data. This is of importance in connection with the use of the crop for hay. The high content of the stems in potassium and nitrogen is likewise apparent. The very high calcium content of the leaves suggests an important

TABLE 7  
*Composition of sweet clover roots at 0-7 and 7-40 inches*

Pounds per ton on water-free basis

ELEMENT	SAMPLE 1		SAMPLE 2	
	Root depth in inches			
	0-7	7-40	0-7	7-40
Nitrogen.....	64.80	67.00	67.20	64.00
Phosphorus.....	5.28	5.12	5.78	4.64
Sulfur.....	5.32	5.00	5.78	5.26
Potassium.....	13.34	11.60	12.00	12.36
Calcium.....	7.20	7.60	6.40	7.20
Magnesium.....	5.32	5.44	4.48	5.12

TABLE 8  
*Composition of sweet clover tops (second year's growth) as influenced by soil treatment*

	POUNDS PER TON WATER- FREE BASIS	INCREASE FOR TREATMENT	
		<i>pounds</i>	<i>per cent</i>
Calcium content on limed land (Urbana).....	40.00		
Calcium content on unlimed land (Urbana).....	31.00	9.00	29.0
Phosphorus content on phosphated land (Exp. fields)...	8.34		
Phosphorus content on unphosphated land (Urbana)....	5.64	2.70	48.9
Potassium content on potassium treated land (Urbana) ..	47.40		
Potassium content on non-potassium treated land (Urbana).....	28.60	18.80	65.7

function of this element in the metabolism of sweet clover and has been already discussed. The leaves of alfalfa were reported by Ames and Boltz as containing 30.4 pounds of calcium on the basis of the figures in table 6. Our results show 57.6 and 44 pounds respectively for fall and summer samples. Calcium is present in the seed in very high amounts. This is unusual for this element, but is in accord with its exceptionally high content in the leaves, and it may bear a relation to some special function not yet studied in sweet clover.

Magnesium is present in the smallest amount in the seed and is practically the same, as the above authors reported for alfalfa seed.

Sulfur is contained in the leaves, seed, stems, and roots in larger amounts than phosphorus. Sulfur may be tolerated in the leaves, but it could hardly be expected to be tolerated to any large extent in the seed. It seems that sulfur, as well as calcium, needs more study from the new standpoint of its relation to the plant metabolism.

#### COMPOSITION OF ROOTS AT DIFFERENT DEPTHS

In table 7 analyses of root samples taken in the fall at 0 to 7 and 7 to 40 inches are included. The root composition in the two layers does not differ beyond the error of sampling. This is evidence that the roots at considerable depth maintain the same percentage composition as they do near the surface.

#### INFLUENCE OF SOIL TREATMENT ON COMPOSITION OF SWEET CLOVER

In the course of various investigations, data showing the influence of soil treatment on the composition of sweet clover tops have been obtained. In table 8, some of these results are presented.

In the calcium comparison, both samples came from brown silt loam, one treated with limestone and the other untreated. Another analysis made, but not included in table 8, was a composite from six experiment fields and shows 37.14 pounds of calcium per ton, where five of the six fields are located on what was originally very poor soil. The samples from phosphated land came from soil which was untreated with potassium and included a composite of six experiment fields, whereas the unphosphated sample grew on soil naturally richer in all elements than five of the six fields from which the composite was made. The potassium comparison samples came from brown silt loam fields, located near each other. The large intake of potassium, where it was applied, is important, and may account in part for the beneficial effects sweet clover produces on other crops when it is plowed under. On a number of the Illinois experiment fields where potassium is applied the sweet clover is decidedly improved. The increases in the corn crop produced by sweet clover on the potassium plots are traceable to the influence of the potassium, perhaps as much as to the nitrogen of the sweet clover. The potassium applied is certainly not lost in the open soils to the deep-rooting sweet clover. The calcium, potassium, and phosphorus content of sweet clover roots may be influenced by soil treatment in the same manner as other crops. The sulfur analyses from Spring Valley may be considered as representing a sulfur-treated sample, and may be compared with those in the other tables. When so compared, here also an influence is evident from the treatment.

It is doubtful if any other factor is important when compared with that of soil treatment for this hardy crop. Inoculation is understood to be in the nature of treatment from a fertility standpoint. The nitrogen content of inoculated sweet clover has been found to be very high from many different soils.

## SWEET CLOVER COMPOSITION COMPARED WITH CORN AND WHEAT PLANT-FOOD REQUIREMENTS

The plant-food contained in sweet clover tops and roots, at the time it would be plowed as a green manure, is calculated on an acre basis from known ratios of roots to tops, and from known weights per acre. These are shown in table 9 and are taken from the May 10 sample of table 5. On untreated normal corn belt soil, sweet clover is deficient in phosphorus, and in potassium, as measured by crop requirements. The large response to these elements where rock phosphate and potassium salts have been applied supports the view that the success of sweet clover may be retarded by a deficiency of these elements in many soils. If the analysis of the samples from the experiment fields, where rock phosphate has been applied, and from the North Farm at Urbana, where potassium has been applied, are measured by crop requirements, then sweet clover would carry sufficient potassium for a corn crop with 20 pounds to

TABLE 9  
*Sweet clover composition compared with wheat and corn plant-food requirements*

ELEMENT	2600 POUNDS TOPS	3100 POUNDS ROOTS	TOTAL POUNDS	PLANT-FOOD REQUIRED BY	
				100 bushels corn	50 bushels wheat
Nitrogen.....	108.7	110.7	219.4	150.0	96.0
Phosphorus.....	7.3	5.7	13.0	23.0	16.0
Sulfur.....	13.3	13.2	26.5	15.3	11.8
Potassium.....	36.0	17.8	53.8	71.0	58.0
Calcium.....	40.4	14.4	54.8	22.0	11.0
Magnesium.....	12.3	12.2	24.5	17.0	8.0

spare, but would still fall short in phosphorus about 4 pounds for the corn, although exceeding the requirements for wheat.

Although it is true that the crops of sweet clover being produced are the basis of the figures used, and the corn crops raised are much lower than the standards employed here; such a relation is no excuse for applying actual average yields, as, in many cases where sweet clover is being used, 100 bushels of corn and 50 bushels of wheat are not too high to expect, and these figures may need to be raised for future study with improved conditions of production.

Possibly 85 to 90 per cent of the fertility elements in the sweet clover becomes available to the succeeding crops. In such a case the margin of safety of available phosphorus would not be large even with soil treatment. The figures emphasize the need for phosphate applications before seeding the legume and when it is plowed under. Potassium may be applied with best results prior to seeding.

Attention to meeting the fertility demands of legumes used in soil improvement must not be overlooked, otherwise production of succeeding crops may be limited through deficiencies existing in the green manure.

Sweet clover, although a good ravager, is also a good feeder, responding well when fed. These two characteristics open the way for attaining the highest composition in the important elements for either soil improvement or feed. Where a rapid improvement of soil is sought, limestone, phosphorus, and potassium should be applied on many soils for this crop.

#### SUMMARY

The composition of sweet clover tops and roots has been determined at various periods in its entire growth. The tops decrease in nitrogen, phosphorus, and sulfur with maturity the first season. The roots increase in nitrogen and apparently in other elements while increasing in dry matter. In the second season the roots increase slightly at first and then decrease somewhat parallel to the decrease in the tops which occurs at the same time. These changes are regarded as due in part to translocation, although the evidence is not sufficient for final proof.

Nitrogen is the outstanding element in the composition of both the tops and roots at most periods when the samples were taken. A high percentage of the total nitrogen is found in a soluble form in the roots.

The mineral elements are present in quantity, generally in the following order: potassium, calcium, magnesium, sulfur, and phosphorus.

Potassium appears to be required in relatively large amounts by this crop. A large response to potassium feeding has been noted. A high content, suggesting a large requirement for some special metabolic processes, was found at several periods.

Calcium is also demanded in large amounts for sweet clover. All its functions are not known, but it would appear that it is either needed in larger amounts for its known functions or that it fills a special and as yet unrecognized place in the metabolism of sweet clover. It is present both in the seed and in the leaves in excess of other elements except nitrogen.

Magnesium is present in normal amounts in the seed, being about one-third that of the phosphorus content. It is very high in the leaves, being two to three times that of the phosphorus content.

Sulfur is present in sweet clover in large amounts from a relative viewpoint. Out of 47 comparisons with phosphorus it exceeds that element in 42 cases. It is high even in the seed, and very high in the leaves. It is not nearly so high in the seed in proportion to phosphorus as it is in the leaves.

Phosphorus was found in the smallest amounts of all elements studied. It was higher in the fall roots than in other samples. Applying this element for the use of the growing sweet clover and again when it is plowed under offers an excellent opportunity to make it more available for succeeding crops.

The composition of the roots is nearly constant at the depths studied.

The composition of sweet clover may be considerably influenced by applications of limestone, phosphorus, and potassium.

Fall plowing, although adding much organic matter and nitrogen, will not add as much material as spring plowing, if the crop has made as much growth as possible before the usual time of plowing for corn.

The extensive root system existing in late fall and early spring affords an opportunity, if plowed at these periods, to enrich deeper layers than the surface soil. Either fall or spring plowing of this crop will be highly beneficial because of its large tonnage per acre and its relatively high content of nitrogen, potassium, calcium, and sulfur.

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# A STUDY OF PHYSIOLOGICAL BALANCE FOR ALFALFA IN SOLUTION CULTURES<sup>1</sup>

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Physiological balance investigations on plants have been carried on for several years by various workers. The solution culture method lends itself very conveniently to this kind of study, especially since this method has become systematised through the work of Tottingham (7) and of Shive (6). Studies of the ratios of the elements known to be of importance in the plant economy, have by no means lost in interest. While Loew's (2) theory of the calcium-magnesium ratio is still being contested in some quarters, other students of the question are convinced that ratio relationships play a vital part in plant nutrition. Lemmermann and Einecke (1) for instance, conclude on the basis of their experiments, that there must exist optimal ratios not only between calcium and magnesium, but between the other nutrients as well.

Cereals and soybeans stand out prominently among the plants experimented with in physiological balance studies. Alfalfa, properly handled, can be grown in solution cultures for a considerable length of time—the writer (3) has grown one series of plants in solution cultures for 98 days—it can also possibly be made to yield more than one crop, and thus afford the benefit of a more extended consecutive period of observation and study. If, in addition, the great economic importance of this legume is considered, the incentive offered by it for this kind of study is obvious.

## METHOD OF PROCEDURE

The alfalfa seed used in the present work came from a lot which furnished the seeds for some previous studies with alfalfa in solution cultures (3, 4) and in soils. The technique for germination, transfer to net and to jars, renewal of solutions, measurements of water absorption and supply of iron, harvesting, drying, and analysis, was the same as described in connection with those studies.

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<sup>2</sup> Thanks are due Dr. John W. Shive, of the department of plant physiology, for his continuously encouraging attitude and for his valuable suggestions in connection with the present work.

Twenty-one solutions in duplicate, in jars with 3 plants to a jar, were used in the present series, and the culture solution  $R_6C_2$  (Shive, 1.75 atm.), in triplicate, served as a check. The seedlings were transferred from the germination dish to the seedling net on August 20, 1923, two days after sowing. On August 29, the plantlets were transferred to the jars in which they were grown until November 14, 1923.

The type of solutions used in this experiment is that designated as type "T" in a plan for coöperative research on the salt requirements of representative agricultural plants prepared for a special committee of the division of biology and agriculture of the National Research Council (5). All the solutions had a

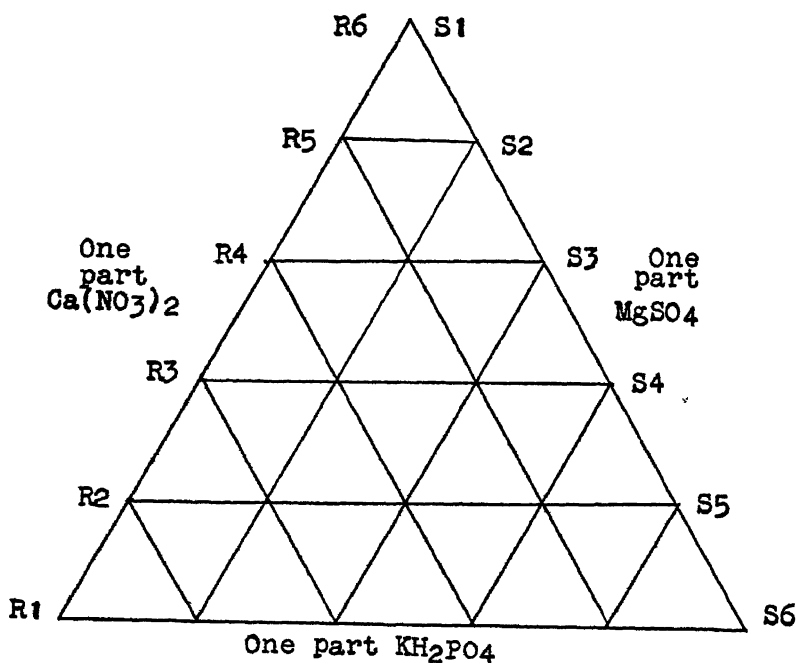


FIG. 1. DIAGRAM SHOWING SOLUTION NUMBERS AND VOLUME-MOLECULAR PROPORTIONS OF THE THREE SALTS

total concentration of one atmosphere possible osmotic pressure. These 21 solutions, as explained in the publication referred to, are arranged in such a manner as to give all possible combinations, in variations of one-eighth of the total volume-molecular concentration of the three salts—monopotassium phosphate, calcium nitrate, and magnesium sulfate—which make up the solutions. A triangular diagram as shown in figure 1, with lines running parallel to the three sides, respectively, is laid off so that each point of intersection represents a different solution. These points, beginning with the one at the lower left hand ( $R_1S_1$ ), and running to the right in the row, with the rows running from

the base to the apex of the triangle, are designated by the solution numbers in the order arranged in table 1.

The partial volume-molecular concentrations and the molecular proportions of the solutions used, as well as those of the check solution, are given in table 1.

TABLE 1

*Partial volume-molecular concentrations and molecular proportions of  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$ , used in the culture solutions*

Total osmotic value, approximately 1.00 atmosphere, except in the check, which is 1.75 atmospheres.

SOLUTION NUMBER	PARTIAL VOLUME-MOLECULAR CONCENTRATIONS			MOLECULAR PROPORTIONS		
	$\text{KH}_2\text{PO}_4$	$\text{Ca}(\text{NO}_3)_2$	$\text{MgSO}_4$	$\text{KH}_2\text{PO}_4$	$\text{Ca}(\text{NO}_3)_2$	$\text{MgSO}_4$
$\text{R}_3\text{C}_2$ , check	0.0180	0.0052	0.0150	3.77	1.09	3.14
$\text{IR}_1\text{S}_1$	0.0027	0.0027	0.0161	1	1	6
$\text{S}_2$	0.0025	0.0049	0.0123	1	2	5
$\text{S}_3$	0.0024	0.0071	0.0094	1	3	4
$\text{S}_4$	0.0022	0.0089	0.0067	1	4	3
$\text{S}_5$	0.0022	0.0108	0.0043	1	5	2
$\text{S}_6$	0.0020	0.0122	0.0020	1	6	1
$\text{R}_2\text{S}_1$	0.0053	0.0027	0.0132	2	1	5
$\text{S}_2$	0.0049	0.0049	0.0099	2	2	4
$\text{S}_3$	0.0047	0.0071	0.0071	2	3	3
$\text{S}_4$	0.0045	0.0090	0.0045	2	4	2
$\text{S}_5$	0.0041	0.0104	0.0021	2	5	1
$\text{R}_3\text{S}_1$	0.0076	0.0025	0.0101	3	1	4
$\text{S}_2$	0.0072	0.0048	0.0072	3	2	3
$\text{S}_3$	0.0068	0.0068	0.0045	3	3	2
$\text{S}_4$	0.0065	0.0086	0.0021	3	4	1
$\text{R}_4\text{S}_1$	0.0099	0.0025	0.0074	4	1	3
$\text{S}_2$	0.0094	0.0047	0.0047	4	2	2
$\text{S}_3$	0.0090	0.0068	0.0022	4	3	1
$\text{R}_5\text{S}_1$	0.0123	0.0024	0.0049	5	1	2
$\text{S}_2$	0.0118	0.0047	0.0023	5	2	1
$\text{R}_6\text{S}_1$	0.0145	0.0024	0.0024	6	1	1

## DISCUSSION OF RESULTS

The relative values for dry weights, water absorption, water requirement, and nitrogen content, separately for tops, roots, and whole plants, as compared with the check taken as 100.0, and the actual figures for the check, are given in table 2. The actual values for all these data are presented graphically in figure 5, the cultures in this presentation being arranged in order of the highest

TABLE 2  
*Relative dry weights, water absorption, water requirements, and nitrogen content of tops, roots and whole plants, of alfalfa grown 77 days in solution cultures of type "I," from August 29 to November 14, 1923*

Figures in parenthesis are actual values

SOLUTION NUMBER	YIELD			WATER REQUIREMENTS			NITROGEN, PER CENT			NITROGEN, GRAMS		
	Tops	Roots	Whole plants	Water absorption	Tops	Roots	Whole plants	Tops	Roots	Whole plants	Tops	Roots
R <sub>0</sub> C <sub>1</sub> check	100.0 (3.895)	100.0 (1.352)	100.0 (5.247)	100.0 (2,227)	100.0 (576.6)	100.0 (1,652.6)	100.0 (426.6)	100.0 (3.36)	100.0 (3.03)	100.0 (3.29)	100.0 (0.1317)	100.0 (0.0410)
IR <sub>1</sub> S <sub>1</sub>	103.0	90.6	99.8	108.7	104.8	120.8	108.6	110.7	85.5	105.5	113.9	77.0
S <sub>1</sub>	105.8	84.6	100.4	115.2	108.0	130.8	114.2	115.3	89.1	110.6	122.1	75.3
S <sub>2</sub>	116.8	102.5	113.1	114.3	97.1	112.1	97.8	111.5	76.9	104.2	130.2	78.8
S <sub>4</sub>	112.5	89.3	106.5	115.8	101.9	129.3	108.0	109.1	85.1	104.8	122.7	76.1
S <sub>6</sub>	132.4	97.6	123.6	132.6	100.0	141.1	108.1	111.8	85.8	107.6	148.0	83.6
S <sub>8</sub>	108.2	76.5	100.0	102.3	93.7	135.0	101.8	113.0	90.4	109.7	122.3	68.8
R <sub>2</sub> S <sub>1</sub>	83.8	69.3	80.0	89.0	105.6	127.9	110.7	113.6	84.4	107.2	94.3	58.5
S <sub>4</sub>	101.6	97.7	100.6	107.0	104.8	112.8	105.9	105.9	92.0	102.7	107.6	90.0
S <sub>6</sub>	119.1	108.5	116.4	115.4	97.1	105.0	99.3	110.3	96.0	107.2	131.4	104.1
S <sub>8</sub>	134.9	103.1	126.7	137.9	101.3	133.7	108.2	114.5	80.5	108.5	154.5	82.9
S <sub>1</sub>	94.7	82.0	91.4	93.2	98.4	113.2	101.8	107.9	85.5	103.3	102.2	70.0
R <sub>2</sub> S <sub>1</sub>	99.1	82.6	94.9	96.1	97.3	116.1	101.0	108.8	94.0	106.3	107.8	77.5
S <sub>1</sub>	122.6	92.7	114.9	124.2	99.8	132.5	106.8	109.1	97.3	107.3	133.9	90.2
S <sub>2</sub>	97.8	89.4	95.6	107.9	107.3	117.1	109.9	104.7	80.1	99.3	102.4	71.7
S <sub>4</sub>	89.4	75.5	85.8	96.4	106.9	122.7	112.0	108.5	86.1	104.2	97.0	64.9
R <sub>2</sub> S <sub>1</sub>	109.2	85.3	103.0	111.9	101.4	130.8	107.8	112.4	96.7	109.7	122.7	82.4
S <sub>2</sub>	99.8	78.2	94.2	121.1	121.3	154.1	128.7	103.8	91.0	101.5	103.6	71.2
S <sub>4</sub>	81.5	85.2	82.4	84.3	99.8	99.2	99.4	102.9	94.0	100.6	83.9	80.2
R <sub>2</sub> S <sub>1</sub>	87.6	61.7	80.9	84.6	96.3	141.1	104.9	111.8	89.7	108.5	97.9	55.3
S <sub>2</sub>	107.4	91.2	103.2	99.7	92.1	112.5	95.9	102.3	86.1	99.3	109.9	78.5
R <sub>2</sub> S <sub>1</sub>	100.9	72.8	93.7	96.6	95.6	133.2	103.1	107.9	107.2	108.5	109.0	78.0

yields of tops. The areas representing the highest 7 yields, separately for tops, roots, and whole plants, are outlined on the triangular diagrams (figs. 2, 3, and 4).

### Yields

It will be noted that the points on the triangle (fig. 2) representing the solutions producing the highest 7 yields of tops, are comprised within one continuous area, by far the greater part of which, covering 5 out of the 7 solutions, lies in the region characterized by a rather high molecular proportion of calcium nitrate. The lowest of this group of high top yields—solution  $R_4S_1$ —has<sup>u</sup> the

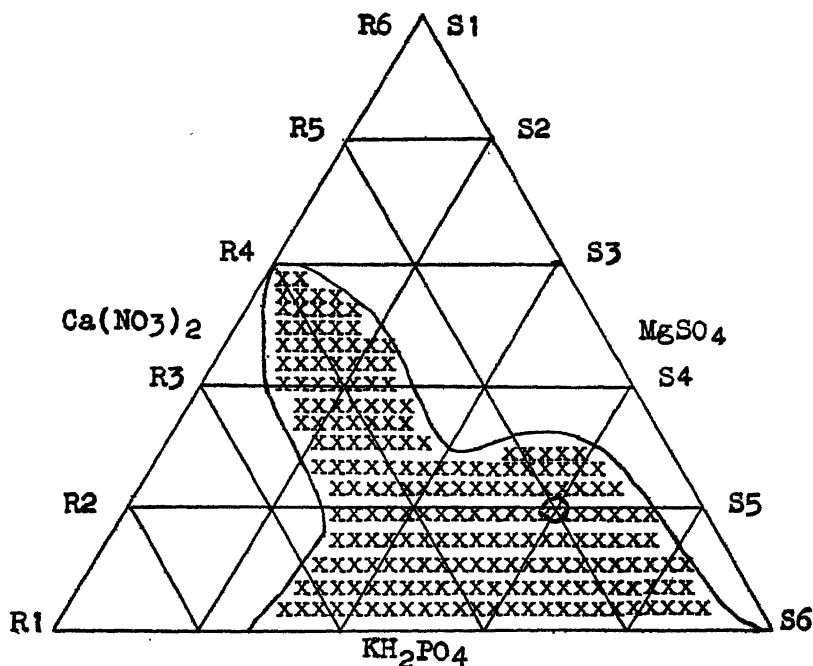


FIG. 2. DIAGRAM SHOWING POSITION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF TOPS

lowest molecular proportion of calcium nitrate in the group. The two solutions  $R_2S_4$  and  $R_1S_6$ , producing respectively the highest and the next highest top yield of this group, have volume-molecular proportions of 2-4-2 and 1-5-2, respectively, for monopotassium phosphate, calcium nitrate and magnesium sulfate. As seen from table 2, compared with the top yield of the check,  $R_5C_2$  (Shive, 1.75 atm.), taken as 100.0, the values for solutions  $R_2S_4$  and  $R_1S_6$ , are 134.9 and 132.4, respectively.

When the values for root yields are considered, it will be noticed, that the point representing the solution,  $R_2S_8$ , which produced the highest yield, is shifted on the triangle, in the same row, one place to the left of that representing

the solution,  $R_2S_4$ , giving the highest top yield. The volume-molecular proportions of  $R_3S_3$ , are 2-3-3 for monopotassium phosphate, calcium nitrate, and magnesium sulfate, respectively, as against 2-4-2 for solution  $R_2S_4$ , which produced the highest top yield. Solution  $R_3S_3$ , with molecular proportions of 5-2-1 for the salts indicated, is included in the group of the seven solutions producing the highest root yield. This solution, which is high in monopotassium phosphate and low in magnesium sulfate, produced one of the highest top yields in the group of seven which gave medium yields of tops.

The configuration of the area on the triangle, representing the region of

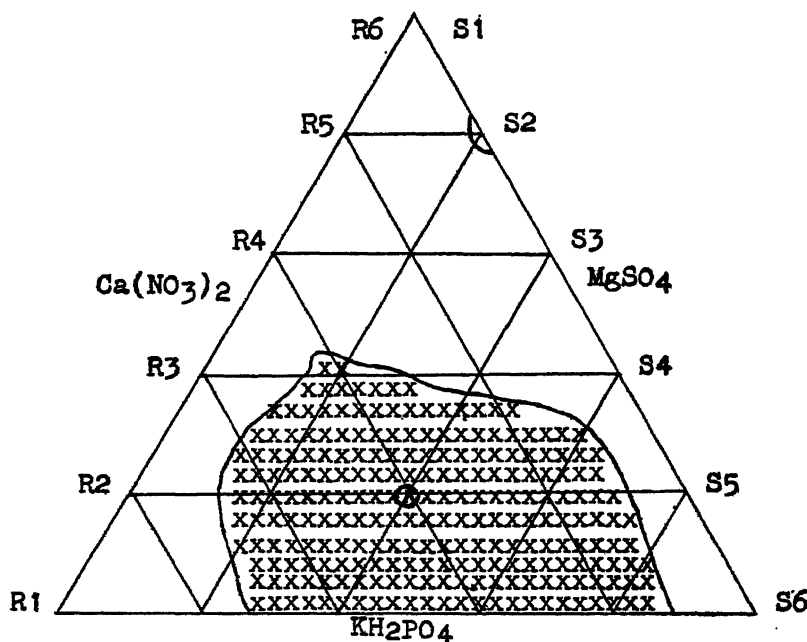


FIG. 3. DIAGRAM SHOWING POSITION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF ROOTS

The highest yield in the group is marked with a circle

highest yield of whole plants is in general the same as that representing the region of highest top yield, except that solution  $R_4S_1$ , with molecular proportions of 4-1-3, is left out and its place taken by solution  $R_3S_3$ , with molecular proportions of 5-2-1; this last solution, as already stated, produced one of the highest medium yields of tops, and also gave a high root yield. The point representing the solution producing the highest yield for the whole plant, is the same as that representing the solution producing the highest yield for tops.

From figure 5, it is seen that the short line to the left, representing the top yield of the check solution,  $R_3C_2$  (Shive, 1.75 atm.), falls below all but 8 of the 21 solutions of type "I," represented on the curve for top yields. Of these 8,

the values for 2 are substantially the same as that for the check. On the other hand, all the points but 3, on the curve representing the root yield of those 21 solutions, fall below the line that corresponds to the check. In the curve representing the whole plant yield of the 21 solutions, 9 fall below the line of the check. In general, on account of the relatively higher root yield of the check, the divergence in the yield values of tops in favor of the solutions of type "I" as compared with the check, becomes somewhat lessened when the corresponding values for the whole plants are compared. There is some difference in the average ratios of root yield to that of whole plant, between the solutions of

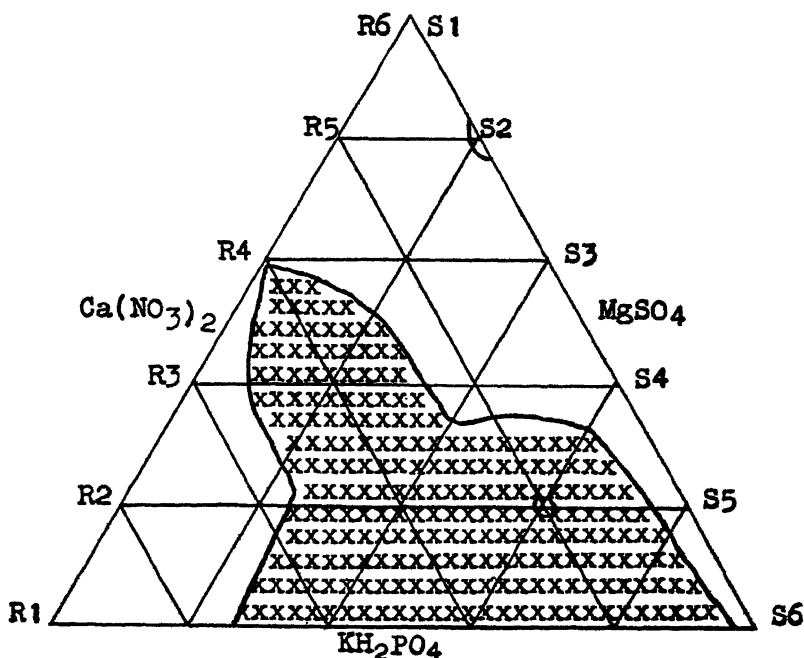


FIG. 4. DIAGRAM SHOWING POSITION OF CULTURES PRODUCING THE HIGHEST SEVEN YIELDS OF WHOLE PLANTS

The highest yield in the group is marked with a circle

type "I" and the check solution. The average value of this ratio for the former is 0.22, for the latter it is 0.26; or, expressed on the basis of the check ratio taken as 100, that of the solutions of type "I" will have the value of 85.

It should be mentioned that a series of cultures like the one here discussed had been conducted previously as a preliminary experiment during a short period of growth, but no detailed study was made of the results. It was noted, however, that the highest yield of tops, of roots, and consequently also of whole plants, was produced by solution  $R_2S_4$ , the same solution that gave the highest yield in the present series.



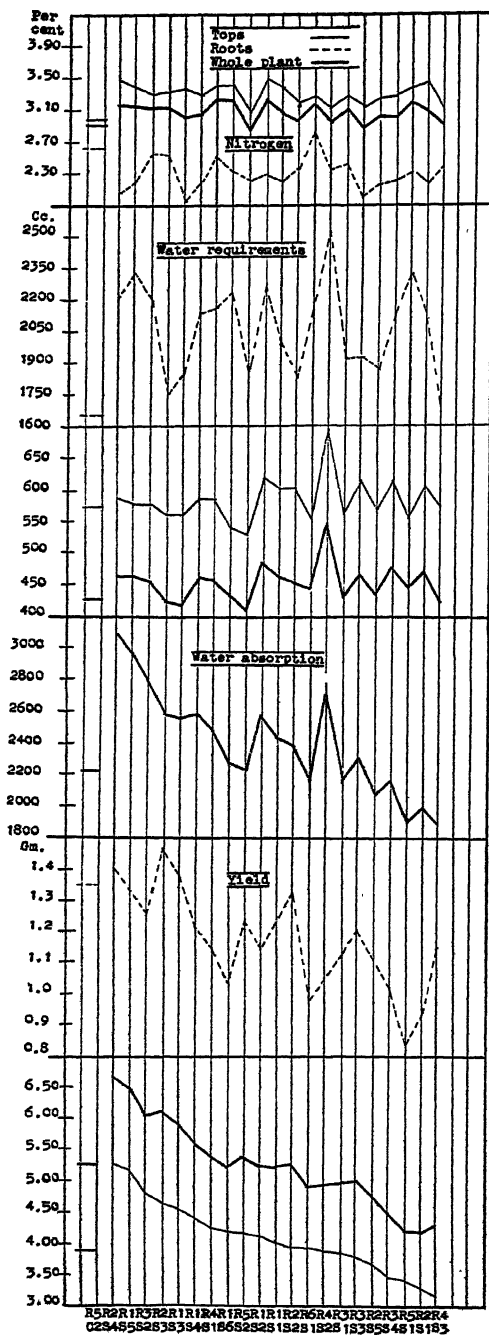


FIG. 5. GRAPHIC SUMMARY OF DATA DETERMINED  
Checks are indicated by short horizontal lines to left

*Water absorption and water requirements*

Except for 8 solutions, which produced a lower total yield than the check, the series of solutions of type "I" showed a higher value for water absorbed than the check. Within the series itself, there is noted a tendency for the curve of water absorption to follow, in a general way, the curve of top yield. An unusually high absorption of water occurred in case of solution  $R_4S_2$ , with a molecular proportion of 4-2-2, for the three salts in the order usually given.

In studying the values for water requirements (cubic centimeter liquid absorbed per gram of dry matter produced), it is noticeable, that from the point of view of top production, there is a tendency for greater efficiency in the utilization of the water absorbed, as the yield goes up higher. The average relative water requirement value for tops for the group of 9 solutions with relative top yield above 107, is 98.2, whereas the average relative water requirement value for the other 12 solutions, goes up to 103.8.

It will be noted further, that although there is in general no great variation in the water requirement values for tops between the check solution and the others, a decided difference is seen to exist when the water requirements of the roots are considered. Of the 21 solutions of type "I," 20 show water requirement values for roots ranging between 105 and 154 as compared with 100 for the check. This high water requirement for roots of the solutions of type "I," naturally brought the average water requirement values of those solutions for whole plants above that of the check. All this is related to the fact mentioned, that the check solution, whether on account of its higher concentration, or because of the particular distribution of the proportions of the tree salts in that solution, is conducive to a relatively greater root development of alfalfa, whereas the solutions of type "I," favor a relatively higher development of tops.

*Nitrogen content*

Notwithstanding the fact that the check solution contained a higher concentration of calcium nitrate than 11 out of the 21 solutions of type "I," the nitrogen percentages of the tops from these latter solutions are invariably higher than that of the tops from the check solution. This appears somewhat remarkable if it is kept in mind that the solutions of type "I" produced on the average higher top yields than the check solution. Precisely the reverse relationship is observed with respect to the nitrogen content of the roots. In this case, all solutions but one of type "I," produced roots with a lower nitrogen percentage than did the check solution. The check solution, it should be remembered, produced at the same time a higher average root yield than that produced by the other solutions. The nitrogen content of tops and of roots in the present case was apparently not governed by the usually accepted principle that a high percentage may be expected to go with a relatively high supply of that element in the medium, or with a low crop yield.

Because of the relatively greater proportion of tops to roots in the whole plant, and to the higher percentage of nitrogen in the tops, the values for the nitrogen percentage in the whole plants of the solutions of type "I," continue on the average to lie above that of the whole plants of the check solution.

The results obtained with the solutions of type "I" in this investigation, with respect to the growth of alfalfa during the first 77 days, may be summarized as follows:

1. The highest yield for tops as well as for whole plants, was obtained with solution  $R_3S_4$ , with molecular proportions of 2-4-2, and a partial volume-molecular concentration of 0.0045, 0.0090 and 0.0045, for monopotassium phosphate, calcium nitrate and magnesium sulfate, respectively. This solution produced a top yield of 134.9 and whole plant yield of 126.7, as compared with top and whole plant yield, respectively, of the check,  $R_4C_2$  (Shive, 1.75 atm.), taken as 100.0

2. The highest root yield was obtained with solution  $R_3S_8$ , characterized by molecular proportions of 2-3-3, and partial volume-molecular concentration of 0.0047, 0.0071 and 0.0071, respectively, for the three salts indicated. This solution produced a root yield of 108.5 as compared with the root yield of the check taken as 100.0. The regions on the three triangles representing, respectively, the solutions producing highest yields of tops, roots, and whole plants, differ only a little from one another.

3. The average for top yields is higher for the solutions of type "I" than for that of the check. The reverse holds true with respect to root yield. This would indicate that the solutions of type "I" are conducive to a relatively greater top growth than is the check solution.

4. A tendency was shown in the solutions of type "I" toward a greater economy in the use of water as the yields went higher.

5. There was not much difference in the water requirements for tops, between the solutions of type "I" and that of the check.

6. The water requirement values for roots, of the solutions of type "I," are considerably above that of the check.

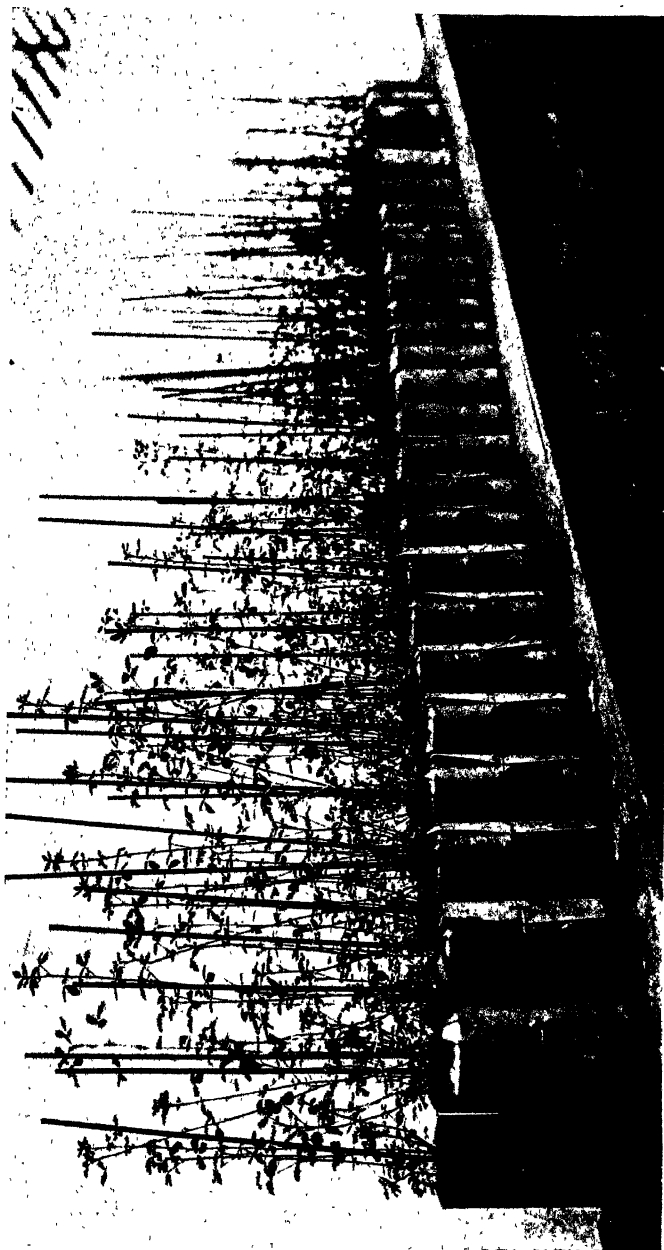
7. The nitrogen percentage is higher in the tops from the solutions of type "I," than in those from the check solution.

8. The nitrogen percentage is lower in the roots from the solutions of type "I," than in those from the check solutions.

9. The nitrogen percentage of the whole plants from the solutions of type "I," is higher than in those from the check solution.

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ALFALFA FIVE WEEKS OLD, GROWN IN SOLUTION CULTURES



# FIXATION OF CALCIUM-MAGNESIUM FROM BURNT LIMES, LIMESTONE AND DOLOMITE INCORPORATIONS IN TWO SOIL ZONES

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Frear (3) pointed out that the beneficial effects of limestone particles upon unfavorable acid soil conditions prevail only after the disintegration of the limestone. It is also true that the maintenance of the ameliorated conditions thus brought about is dependent upon the nature of the absorption complexes and upon the tenacity with which absorbed calcium or magnesium is held against hydrolytic disintegration and leaching. It is likewise apparent that the addition of caustic forms of alkali-earths to a soil containing an abundance of hydrated silica will result (11, 12) at first in the formation of at least some normal silicates, compounds which are hydrolyzed much more readily than the more complex and ultimate alumino-silicates. The response of absorbed calcium-magnesium to hydrolysis influences (a) future supplies of plant nutrients, (b) solubility of amphoteric elements, (c) adaptation of the soil as a medium for bacterial activities, and (d) neutralization of the products formed by such activities.

In the absence of plant growth, the measure of the fixation of a definite alkali-earth carbonate addition is the *difference* between the amount added and the amount accounted for jointly by (a) the disintegration of added, or derived, carbonates and by (b) the amount of alkali-earth outgo, during a given time. This paper records the fixation of added alkali-earths extant at the end of four years, as such fixation was influenced by form, fineness, and zone-of-incorporation in outdoor lysimeters. The Ca-Mg fixation results are given in terms of pounds of  $\text{CaCO}_3$  per 2,000,000 pounds of soil, and as per cent of addition, and represent the differences between a constant equivalent of alkali-earths and the several amounts accounted for jointly by carbonate disintegration and by leaching from surface-zone and subsurface-zone incorporations.

## EXPERIMENTAL

The liming materials used were hydrated high-calcic lime, burnt dolomite, a corresponding mixture of separately calcined  $\text{CaO}$  and  $\text{MgO}$ , and four

<sup>1</sup>The results were obtained by means of a fellowship endowment maintained by the National Lime Association and equipment donated by the American Limestone Company. Previous acknowledgment has been made of assistance rendered by sometime Fellows.

separates and separate-composites of limestone and of dolomite, in a constant equivalence of 3570 pounds of  $\text{CaCO}_3$  (2000 pounds of  $\text{CaO}$ ) per 2,000,000 pounds of fallow soil, moisture-free basis. Analysis of soil and alkali-earth additions, make-up of the washed separates, and details of treatment have been given and the lysimeter installation has been illustrated in a previous contribution (13). An 8-inch stratum of surface soil only was used throughout, and each soil charge rested upon a sand filter bed. In one series of tanks the Ca-Mg additions were incorporated throughout the upper half, or zone, and nothing was added to the lower zone. In a parallel series the additions were incorporated only in the lower zone. Before treatment and placement, the soil was thoroughly mixed to insure uniform composition. After incorporation of Ca-Mg materials the two zones were demarcated by means of asphaltum-coated galvanized iron wire discs. The soil was not stirred during the 4-year period of exposure. No samples of soil were taken from either zone until the end of the 4-year period, so that the results show the *ultimate fate* of the incorporations, but not the speed of the processes responsible for their disintegration and depletion.

#### DISCUSSION

As used throughout the text, the expression *fixation* connotes the final increase in the soil's content of non-carbonate Ca-Mg derived from either (a) the initial direct reaction between soil and hydroxides together with the additional reaction between soil and carbonates which came from such hydroxides or (b) the disintegrations of the several limestone and dolomite incorporations. *Fixation* is not used as synonymous with *absorption*, since the absorbed alkali-earths were subject to the diminishing action of leaching, the results of which are designated as *outgo*. The expression *disintegration* is used to designate the difference between added and residual carbonates, and represents the sum of fixation and outgo.

The persistence of carbonates resulting from alkali-earth additions to the upper and lower zones is given as carbonate increases, as compared with controls, in columns 1 and 6, respectively, of table 1. The difference between the constant addition and each carbonate-residual represents the sum of a fixation and concomitant leaching and is given in columns 2 and 7 for the surface-zone and subsurface-zone incorporations, respectively. The total calcium-magnesium outgo from each tank, corrected for the average obtained from that of the controls, is given in columns 3 and 8 for the upper and lower zones of incorporation, respectively. The fate of each incorporation, as accounted for by fixation, leachings, and residual carbonates, is graphed for the surface and subsurface zones. (Figs. 1 and 2.)

Parallel studies on nitrate and sulfate outgo (7) show that those salts are not retained in appreciable quantities by this soil, when exposed to prevailing rainfall, and they justify the assumption that the bases unaccounted for by the combination of residual carbonate and leaching may be designated as fixed,

TABLE 1  
*Fixation of Ca-Mg from a 3570-pound CaCO<sub>3</sub>-equivalence (2000 pounds CaO) and of Ca(OH)<sub>2</sub>, CaO-MgO, and limestone and dolomite separates in surface-zone and subsurface-zone incorporations with a loam soil under outdoor conditions for a period of 4 years*  
 Results are given in terms of CaCO<sub>3</sub>-equivalence per 2,000,000 pounds of soil, moisture-free basis

TREATMENT	SURFACE-ZONE INCORPORATION					SUBSURFACE-ZONE INCORPORATION					FIXATION FROM SURFACE-ZONE INCORPORATION OVER THAT FROM SUBSURFACE INCORPORATION	
	Carbonate increase	Total CaCO <sub>3</sub> equivalence accounted for by both fixation and leaching	Total Ca-Mg outgo in excess of that from controls	Fixation—Full depth of soil considered		Carbonate increase	Total CaCO <sub>3</sub> equivalence accounted for by both fixation and leaching	Total Ca-Mg outgo in excess of that from controls	Fixation in subsurface zone			
				pounds	per cent				pounds	per cent		
Ca(OH) <sub>2</sub> .....	320	3,250*	271	2,979	83.4 <sup>†</sup>	260	3,310	1,148	2,162	60.5 <sup>‡</sup>	817	22.9 <sup>¶</sup>
CaO-MgO†.....	280	3,290*	231	3,059	85.7	300	3,270	1,215	2,055	57.6	1,004	28.1
CaO-MgO‡.....	280	3,290*	156	3,134	87.8	400	3,170	1,090	2,080	58.2	1,054	29.6
L.S. 10-20.....	3,340	230	78	152	4.3	1,720	1,850	506	1,344	37.6	-1,192	-33.3
L.S. 20-40.....	540	3,030	170	2,860	80.1	340	3,230	817	2,413	67.6	447	12.5
L.S. 40-80.....	80	3,490	211	3,279	91.8	0	3,570	1,098	2,472	69.2	807	22.6
L.S. 80-200.....	200	3,370	226	3,144	88.1	0	3,570	1,133	2,437	68.3	707	19.8
L. S. Comp.§.....	1,300	2,270	51	2,219	62.1	680	2,890	900	1,990	55.7	229	6.4
Dol. 10-20.....	3,416	154	38	116	3.2	3,100	470	153	317	8.9	-201	-5.7
Dol. 20-40.....	1,740	1,830	105	1,725	48.3	1,240	2,330	486	1,844	51.6	-119	-3.3
Dol. 40-80.....	1,020	2,550	152	2,398	66.9	320	3,250	805	2,445	68.5	-47	-1.6
Dol. 80-200.....	300	3,270	207	3,063	85.8	220	3,350	1,013	2,337	65.5	726	20.3
Dol. Comp.§.....	2,080	1,490	115	1,375	38.5	1,400	2,170	689	1,481	41.5	-106	-3.0

\* Assumption of theoretical carbonation.

† Calcined dolomite.

‡ Corresponding mixture of separately calcined CaO and MgO.

§ Equal parts of 10-20-, 20-40-, 40-80-, and 80-200-mesh separates.

¶ Basis of CaCO<sub>3</sub>-equivalence of addition.



chiefly as silicate complexes. The fixations, expressed as pounds and as per cent of the constant  $\text{CaCO}_3$ -equivalence of incorporations, are given in columns 4 and 5 for the surface zone and in columns 9 and 10 for the subsurface zone. The high-calcic and high-magnesian limes were considered as equivalent quantities of carbonates, as justified by previously reported results relative to the carbonation and fixation of  $\text{CaO}$  and  $\text{MgO}$  (5, 8, 12).

Carbonate  $\text{CO}_2$  results established the fact that neither zone enriched the carbonate content of the other; therefore no evidence was adduced to indicate movement of undisintegrated particles resulting from either frost action or gravity. Capillary lift of dissolved bases from the lower zone to the upper zone may be ignored; but it is certain that there was some passage of surface-zone incorporations of  $\text{Ca-Mg}$  and some enrichment of the alkali-earth content of the lower zone as a result of solution and leaching, though such specific fixations by the untreated lower zone are not shown. These could be established quantitatively only by ultimate analyses of both zones, and if the proportion between soil and treatment is considered, it is doubtful whether the time and labor incident to such analyses could be justified by the accuracy which might be anticipated for the results. Moreover, the fixed bases of both zones are within the feeding range of most plants whose roots do not penetrate below the surface soil. Studies are being made, however, of the increase in replaceable bases derived from each incorporation in the treated zone, as compared to that translocated to the adjacent untreated zone.

#### *Fixation from surface-zone incorporations*

**Burnt limes:** The results show that the average fixation from the three types of burnt lime was 85.6 per cent of the addition, when the incorporations were made in the surface zone. When this averaged fixation for the caustic group is supplemented by the small carbonate-residue average, we find that 93.8 per cent of the incorporation is still present after 4 years of fallow exposure. The fixation from  $\text{Ca(OH)}_2$  and that as an average from the two  $\text{CaO-MgO}$  incorporations were, respectively, 11 times and 16 times as great as the corresponding enhancements in  $\text{Ca-Mg}$  outgo. Since the three carbonate residues were practically the same, it is evident that the greater retention shown for the two magnesian limes was due either to the stability of the absorbed magnesium in the upper zone, or to a greater removal of the magnesium salts from the leachings during their movement through the untreated lower zone.

**Limestone:** The meagre fixation of the 10-20-mesh limestone separate in the surface zone is in accord with, and less than, the small disintegration of this separate. With such slight disintegration and fixation, together with the previously reported (7) minimum influence exerted by this separate upon outgo of nitrates and sulfates for the 4-year period, it would seem that no commercial value should be claimed for limestone particles of this coarseness, when they are incorporated in the surface zone without subsequent mechanical transfer to lower depths. The 20-40-mesh product, however, gave a decidedly larger

fixation and also outgo, both values having approximated the corresponding ones from  $\text{Ca}(\text{OH})_2$  and the 40-80-mesh and the 80-200-mesh separates. The composite of limestone separates gave results less than, though most nearly approaching, those from the 20-40-mesh separate. The fixation from each surface-zone incorporation was very much greater than the corresponding enhancement in Ca-Mg outgo. Excluding the slightly disintegrated 10-20-mesh product, the limestone group gave an average fixation of 80.5 per cent of the incorporations, the balance being accounted for by a leaching average of 4.6 per cent and a carbonate-residue average of 14.8 per cent. The average

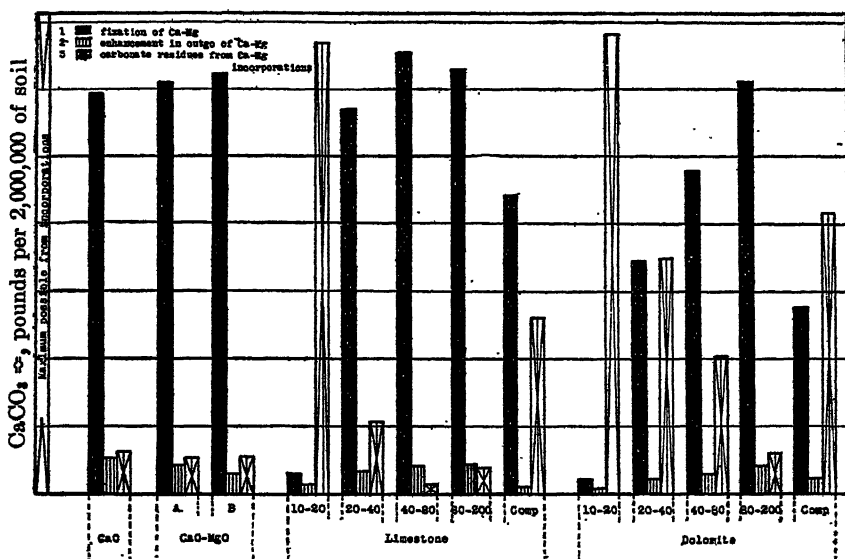


FIG. 1. THE FATE OF SURFACE-ZONE INCORPORATIONS OF  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO-MgO}$ , AND LIMESTONE AND DOLOMITE SEPARATES IN A LOAM SOIL, AT A CONSTANT  $\text{CaCO}_3$ -EQUIVALENCE OF 3570 POUNDS (2000 POUNDS  $\text{CaO}$ ) PER 2,000,000 POUNDS OF SOIL, AS ACCOUNTED FOR BY FIXATION, LEACHING AND CARBONATE RESIDUES AFTER 4 YEARS' EXPOSURE

Sum of 1, 2 and 3 equals the amount of Ca-Mg incorporated  
Burnt  $\text{CaO-MgO}$ : A—calcined dolomite; B—separately calcined  $\text{CaO}$  and  $\text{MgO}$

Ca-Mg fixation from the 20-40-, 40-80-, and 80-200-mesh limestone separates was 15.3 times as great as the corresponding average increase in Ca-Mg outgo through leaching. The average fixation of 86.7 per cent from those 3 separates was 3.3 per cent greater than that from the high-calcic lime.

*Dolomite:* The dolomite separates gave the same relative order in fixation as those of limestone, but the difference between the fixation from each separate and that from the one next in fineness was more marked than in the limestone series. The fixation from each dolomite separate was also less than that from its corresponding limestone separate, but the disparities between corresponding

limestone and dolomite fixations decreased with increase in fineness. This again emphasizes the fact that the more insoluble the limestone—and dolomites are generally less soluble—the finer should be the material applied. The fixation resulting from each surface-zone incorporation of dolomite was decidedly greater than the amount leached and, in the case of the 40-80-mesh and 80-200-mesh separates, the amount of dolomite fixed was materially greater than the undisintegrated residues. Disregarding the 10-20-mesh separate, which suffered very little disintegration, the dolomite group gave an average fixation 14.8 times as great as the enhancement in Ca-Mg outgo from the same group. The maximum fixation from the 80-200-mesh material was practically the same as that from the two magnesian limes.

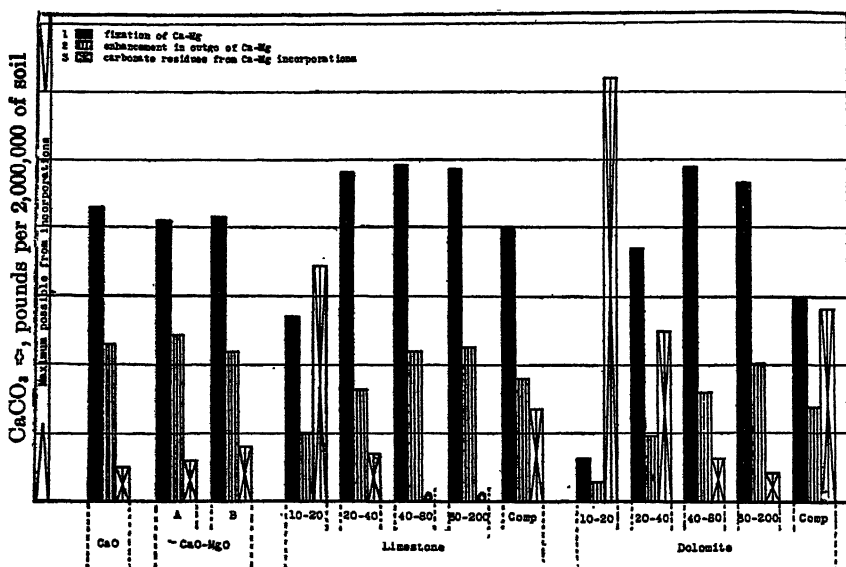


FIG. 2. THE FATE OF SUBSURFACE-ZONE INCORPORATIONS OF  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO-MgO}$ , AND LIMESTONE AND DOLOMITE SEPARATES IN A LOAM SOIL, AT A CONSTANT  $\text{CaCO}_3$ -EQUIVALENCE OF 3570 POUNDS (2000 POUNDS  $\text{CaO}$ ) PER 2,000,000 POUNDS OF SOIL AS ACCOUNTED FOR BY FIXATION, LEACHING, AND CARBONATE RESIDUES AFTER 4 YEARS' EXPOSURE

Sum of 1, 2 and 3 equals the amount of Ca-Mg incorporated  
Burnt  $\text{CaO-MgO}$ : A—calcined dolomite; B—separately calcined  $\text{CaO}$  and  $\text{MgO}$

#### *Fixation from subsurface-zone incorporations*

**Burnt limes:** In the subsurface group the fixations from the three limes were close to 60 per cent, or less than the average of 68.4 per cent from the three closely agreeing finer limestone separates, as accounted for by the larger average outgo and also larger carbonate residues from the three burnt limes. The carbonate residues from burnt limes are due to the unavoidable lumping during

incorporation with the soil and the resultant protective effect of  $\text{CaCO}_3$  coatings over the small lumps, which has been previously stressed (13, p. 414).

*Limestone:* The minimum fixation of limestone came from the 10-20-mesh separate. The disintegrative action of the subsurface zone was of such intensity as to bring the three limestone separates, 20-40, 40-80- and 80-200-mesh, to the same level by the end of the 4-year period. The fixation from the limestone composite again agrees most closely with that from the 20-40-mesh separate. Not including the 10-20-mesh separate, the average limestone fixation was 2.36 times as great as the corresponding enhancement in Ca-Mg content of leachings, whereas a corresponding proportion of 2.40 to 1 obtains from similar averages for the three finer limestone separates. Each of the three finer limestone separates gave a fixation in excess of that from the high-calcic lime.

*Dolomite:* The resistance of the dolomite to disintegration is again well demonstrated by the minimum fixation of the 10-20-mesh separate. The influence of fineness in overcoming this resistant property of dolomite is further demonstrated by the marked increase in fixation from the 20-40-mesh separate over that from the 10-20-mesh product before the end of the 4-year period and also in the final approximation of the 40-80-mesh and 80-200-mesh results to those from the corresponding limestone separates. Not including the 10-20-mesh separate, which underwent such slight disintegration, the dolomite group fixation was 2.71 times as great as the average increase in Ca-Mg leachings, whereas for the 20-40, 40-80, and 80-200-mesh average a corresponding value of 2.88 obtains. The fixation from each of the two finer dolomite separates was greater than that of each of the two high-magnesian limes in the deep incorporations.

#### *Surface-zone versus subsurface-zone incorporations*

The variations between the fixation resulting from surface-zone and subsurface-zone incorporations are given in columns 11 and 12 of table 1 and are shown graphically in figure 3. The fate of each incorporation, as accounted for by non-carbonate fixation, leaching, and carbonate residual, has been given in detail in figures 2 and 3 for upper- and lower-zone incorporations, respectively.

If the two zones were of equal effect in bringing about disintegration of carbonates, it would follow that the greater fixation and conservation of bases would be found where the incorporations were made in the surface zone, for the lower acid zone would retain some of the bases carried by the leachings from the enriched upper zone. The two zones, however, developed distinctly different capacities, or rather speeds, for carbonate disintegration. This greater decomposition in the lower zone may be accounted for by increase in mineral "*acidoids*" after placement, as influenced by greater bacterial activities in the usually more moist second 4 inches (1, 4) and by parallel enhancement in Ca-Mg outgo. Frear (2) was of the opinion that the surface zone is the most

acid stratum of the surface soil, but if true as a periodic variation in cultivated soils, such a variation did not prevail when the present soil was placed, because of thorough mixing. A 1:5 aqueous suspension of the air-dried loam, sampled when placed and then stored for 4 years, gave an electrometric pH value of 5.96, as against pH values of 6.08 and 6.18 for the upper and lower zones of the controls after 4 years of leaching. However, after an extraction immediately preceding the determination (14), these three samples—reserve and upper and lower zones which had been exposed for 4 years—gave corresponding pH values of 7.04, 6.95, and 6.63.

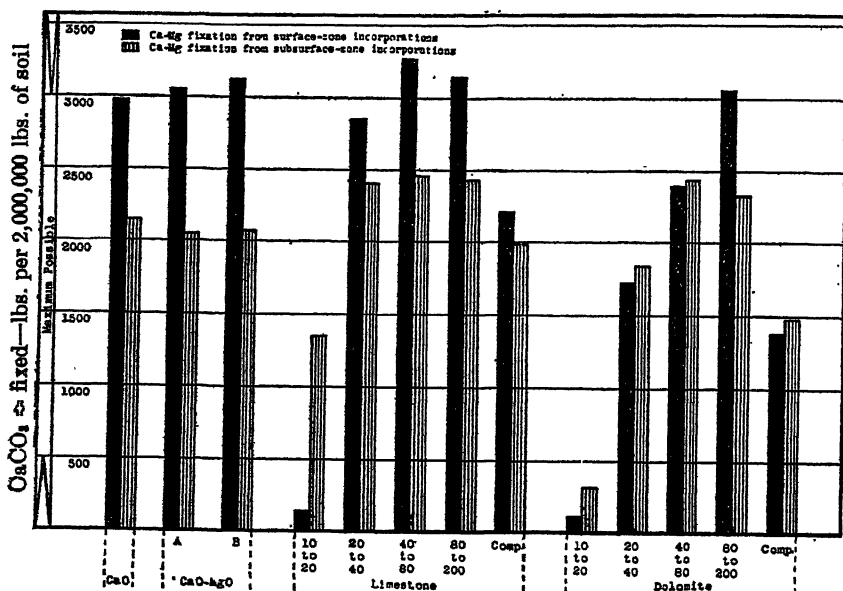


FIG. 3. INFLUENCE OF ZONE OF INCORPORATION UPON THE FIXATION OF CALCIUM-MAGNESIUM FROM  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO-MgO}$ , AND LIMESTONE AND DOLOMITE SEPARATES IN A LOAM SOIL, AT A CONSTANT  $\text{CaCO}_3$ -EQUIVALENCE OF 3570 POUNDS (2000 POUNDS  $\text{CaO}$ ) PER 2,000,000 POUNDS OF SOIL AFTER 4 YEARS' EXPOSURE—TERMS OF  $\text{CaCO}_3$  PER 2,000,000 POUNDS OF SOIL, MOISTURE-FREE BASIS

Burnt  $\text{CaO-MgO}$ : A—calcined dolomite; B—separately calcined  $\text{CaO}$  and  $\text{MgO}$

The 2-zone influence at work in the case of the upper-zone incorporations, as compared with the lower-zone influence alone in the case of the deep incorporations, is responsible for material differences in  $\text{Ca-Mg}$  outgo. The greatest leachings from the subsurface-zone incorporations of limestone came from the two finer separates which suffered complete disintegration, so that it would seem that the lower zone of greater moisture content and greater bacterial activities also gave greater hydrolysis of the absorption complexes.

*Burnt limes:* The average fixation from the three most soluble and active

burnt lime materials was 85.6 per cent of the addition, where both zones restrained outgo of bases, as against a non-carbonate retention of only 58.8 per cent for a similar average where the bases passed directly from the lower zone of incorporation and through the sand filters.

*Limestone:* When incorporated in the surface zone, each of the three finer separates and the composite of limestone showed fixation in excess of that from its analogue in the lower zone. The average of these four fixations in the surface zone was 2876 pounds, or 80.6 per cent of the addition, as against 2328 pounds and 65.5 per cent, respectively, for corresponding averages from incorporations made to the lower zone. In the case of the 10-20-mesh limestone separate, the carbonate disintegration in the lower zone was 8.04 times that in the upper zone and a minus value of 33.3 per cent is obtained in the last column. The carbonate disintegration in the surface zone was only 230 pounds, which was the maximum amount that the subsurface zone could stop for the 4-year period, whereas the disintegration in the subsurface zone was 1850 pounds—506 pounds of which passed into the leachings. But with the finer separates, where the extent of surface-zone disintegration equalled or approached that in the subsurface, the retentive property of the subsurface zone is definitely registered. This untreated surface soil stratum lying below the upper treated zone, thus shows the retardative tendency which was exerted more intensely by its subsoil (9) in stopping large amounts of Ca-Mg from heavy additions, the stoppage by the surface soil, however, being without basic interchange (10). The smallest excess of fixation exerted by both zones over that in the subsurface zone alone is shown for the composite, with the greater values for the separates of lesser size. The calculated average for the composite shows a value of 7.2 per cent, as against the determined value of 6.4 per cent.

*Dolomite:* The same observations made for the minus value for the 10-20-mesh limestone in columns 11 and 12 apply also to 4 of the 5 dolomite additions, which were more extensively disintegrated in, and more extensively leached from, the lower zone. But, the 80-200-mesh dolomite was of such fineness as to offset the less rapid disintegration in the surface zone, so that the carbonate residuals for the upper and lower zones were concordant and only 300 pounds and 220 pounds, respectively. In this case the disintegrated fraction, subject to leaching from the surface zone and through the subsurface zone, amounted to 3270 pounds, as against the 3350-pound disintegration which was subject to outgo from the subsurface zone alone. However, the outgo from the latter was 806 pounds in excess of that from the former. The 20.3 per cent 80-200-mesh dolomite fixation is, therefore, practically identical with that of 19.8 per cent for the corresponding limestone separate.

The Ca-Mg outgo from the surface-zone incorporations of 40-80-mesh dolomite was only 55 pounds less than that from the 80-200-mesh dolomite separate, as a result of the equalizing action of the untreated lower zone; but the carbonate disintegration from the 80-200-mesh separate was 720 pounds

in excess of that from the 40-80-mesh separate. A decided difference in the *speed* of disintegration of these two dolomite separates is thus manifested. However, this difference in speed of disintegration was greatly minimized in the more active lower zone where the differences between their respective carbonate disintegrations and leachings were only 100 pounds and 208 pounds, respectively. With this lack of disparity between outgo of Ca-Mg from lower-zone incorporations of these two dolomite separates, together with the corresponding small and concordant carbonate residuals, it is apparent that only small differences obtained at any time in the amounts of absorbed bases subject to hydrolysis and leaching from the lower zone. As a result of the difference in speed of disintegrations of the two finer separates in the surface zone and the near-equal disintegration in the lower zone, the upper-zone incorporation of the more rapidly disintegrated 80-200-mesh dolomite separate shows a fixation 20.3 per cent in excess of that found for the same separate in the lower zones, as against a minus value of 1.6 per cent in the case of the less rapidly absorbed 40-80-mesh separate.

The fixation from the surface-zone incorporation of  $\text{Ca}(\text{OH})_2$  was 22.9 per cent in excess of that from the lower-zone incorporation, on the basis of the 3570-pounds  $\text{CaCO}_3$ -equivalent addition. This difference is of practical and economic importance. The higher corresponding difference of 28.85 per cent as an average from the CaO-MgO additions, reflects the greater insolubility of the magnesium absorption-complexes. Greater fixations from surface-zone incorporations are also shown for the three finer limestone separates and composite; but the reverse is true of the 10-20-mesh separate. This is accounted for by the fact that the disintegration of the 10-20-mesh separate in the subsurface zone was 8.03 times that of the surface-zone incorporation, whereas their respective ratios between enhancement in outgo and fixation were 1.95:1 and 2.65:1. The curves obtained by plotting Ca-Mg outgo from limestone and dolomite against the fineness of their separates give a line gradually rising with fineness, for both materials and for both zones of incorporation. However, the curves for both materials are more nearly horizontal lines, in the case of the surface zone incorporations. This illustrates the equalizing effect exerted by the lower untreated zone, as offsetting variations in magnitude of fixations and in residual carbonates in the upper zone. In the lower zone there appears a good correlation between fixation and Ca-Mg outgo. This may be taken as indicating that the hydrolysis of absorbed materials served to furnish most of the leached Ca-Mg. Corresponding curves for carbonate residuals give V-shape conformations, with 10-20-mesh separates and composites as terminals. The sides of the curves are much more precipitous in the case of the upper-zone incorporations. The apex of the triangle was more widely spread for the limestone than for the dolomite in the upper-zone incorporations. In the comparison between zones there was a decidedly greater spread at the apex for limestone and for dolomite, in the lower-zone carbonate residues. Disregarding all variations in speed of disintegration and

extent of leachings, the 13 equivalent incorporations in the surface zone gave an average  $\text{CaCO}_3$ -equivalent fixation of 2269 pounds, as against 1952 pounds from subsurface-zone incorporations. If the two 10-20-mesh products be eliminated, the other 11 incorporations give corresponding fixations of 2658 pounds and 2156 pounds, or 74.5 per cent and 60.4 per cent of incorporations for the upper and lower zones of incorporation, respectively. The entire 13 incorporations gave an average fixation 14.6 times as great as the average enhancement in Ca-Mg outgo for the upper-zone incorporations. Excluding the two 10-20-mesh separates, the ratio of fixation to outgo is 15.4:1. Corresponding ratios of 2.29:1 and 2.28:1 are obtained for the 13 separates and 11 separates, respectively, in the case of subsurface-zone incorporations.

As a general conclusion it is established that the surface-zone incorporation of each treatment resulted in a greater Ca-Mg fixation, though less rapid carbonate disintegration, than that found for the corresponding incorporation in the subsurface zone. Conversely, ultimate disintegration and outgo of Ca-Mg from each incorporation were both greater when made in the lower zone. The full depth of soil was effective, however, in the case of the upper-zone incorporations, the upper zone serving as a disintegrating and retaining medium and the lower mainly, if not entirely, as an absorptive stratum for the outgo of Ca-Mg from the overlying treated zone. On the other hand, only the lower zone, or only one-half of the full depth, functioned to effect disintegration and to fix the disintegrated fractions of the incorporations.

#### SUMMARY

Data are given to show the fate of equivalent  $\text{Ca}(\text{OH})_2$ , CaO-MgO, and limestone- and dolomite-separate incorporations in the upper and lower zones of a loam soil after 4 years of outdoor exposure, as accounted for by the final amounts of non-carbonate fixations, leachings, and carbonate residues.

After extraction of soluble salts the lower zone of the untreated and initially uniform soil showed an acidity in excess of that of the upper zone as the result of 4 years of leaching without cultivation.

The Ca-Mg fixation, leachings, and carbonate residues from  $\text{Ca}(\text{OH})_2$ , and two CaO-MgO incorporations in the surface-zone are comparable, whereas their fixations and outgo do not differ greatly from the corresponding ones from the 20-40-, 40-80-, and 80-200-mesh limestone, and the 80-200-mesh dolomite separates. The average of fixations from those 7 incorporations is 3074 pounds, or 14.07 times the 210-pound corresponding loss by leaching. Both 10-20-mesh separates gave only slight fixation and acceleration in Ca-Mg outgo. Each composite of separates agreed most closely with its respective 20-40-mesh separate in extent of fixation. The average fixation of the 13 surface-zone incorporations was 2269 pounds, or 14.6 times a similar 155-pound average for outgo.

In the subsurface-zone series the fixation, leaching, and residual carbonate



results from  $\text{Ca}(\text{OH})_2$  and the  $\text{CaO-MgO}$  incorporations show concordance. The more slowly though completely disintegrated limestone separates of 40-80-mesh and 80-200-mesh fineness show greater fixation and approximately the same outgo as the caustic group. Though not completely disintegrated, the 20-40-mesh limestone and the 40-80- and the 80-200-mesh dolomite gave ultimate fixations in excess of—and leachings less than—the average from the caustic group.

In general the limestone separates were more extensively fixed than their corresponding dolomites, especially in the coarser materials. Except for the 10-20-mesh separates the disparities between limestone and dolomite fixations were not so great as those between leachings and carbonate residues.

The ratios of fixation to outgo for the 13 incorporations were 14.6:1 for the upper zone and 2.29:1 for the lower zone. The supplementary effect of the untreated lower zone served to increase greatly the amount of Ca and Mg fixed from surface-zone incorporations of  $\text{Ca}(\text{OH})_2$ ,  $\text{CaO-MgO}$ , and all of the limestone incorporations, with the exception of the 10-20-mesh separate. The lesser fixation of Ca-Mg in the lower zone is reflected in a correlative increase in outgo. The limestone separates were more extensively disintegrated in the lower zone, but with the exception of the 10-20-mesh separate, the fixation from subsurface-zone incorporations was less than that from incorporations in the upper zone. In the dolomite group the combination of greater disintegration and enhanced outgo gave greater fixation from subsurface-zone incorporations for the composite and all separates except the 80-200-mesh, the exception being attributable to the more rapid disintegration of this separate, as indicated by comparisons of its carbonate-residues and leachings with those of its surface-zone analogue and those of the 40-80-mesh separate from the two zones.

Practical adaptations of the findings are (a) The coarser the separate the deeper should be the incorporation to insure disintegration; (b) Dolomite should be ground finer than limestone; (c) a 20-40-mesh product may be considered as about equivalent to an average 10-mesh product; (d) surface-zone incorporations will give the greatest conservation of alkali-earths.

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# THE ORIGIN AND NATURE OF THE SOIL ORGANIC MATTER OR SOIL "HUMUS": I. INTRODUCTORY AND HISTORICAL<sup>1</sup>

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## INTRODUCTION

The organic matter commonly found in the soil is distinctly different in composition from the tissues of plants and animals which are added to the soil, either in the form of stable or green manures or as various plant and animal residues. The soil organic matter is largely secondary in nature: it consists, on the one hand (*a*) of the constituents of plants and animals which have been introduced into the soil and which are undergoing decomposition, (*b*) of various intermediary products which have been formed under certain environmental conditions and which may be decomposed further, (*c*) of substances which resist decomposition and which may persist in the soil for a considerable time; and on the other hand, of a number of substances that have been synthesized by the numerous groups of microorganisms which, as living and dead cells and as cell derivatives, form the soil population. The soil organic matter is thus found to be genetically a heterogeneous mass of substances, undergoing constant change in composition. It approaches a condition of homogeneity when its composition reaches a certain stage of equilibrium and when it becomes more or less incorporated in the soil. This homogeneous mass is usually spoken of as "humus."

Just what this "humus" is and how it originates in the soil is still largely a matter of conjecture, although various theories—some based on experimental facts—have recently been submitted. These tend to explain the origin if not the nature of this "humus." There is a complete lack of uniformity concerning the very usage of the term "humus." Some investigators (largely European) use the term "humus" to designate the total organic matter in the soil. Frequently no attempt is made to differentiate between the organic matter added to the soil in the form of various organic residues, and the soil organic matter itself, which is distinctly different in composition and decomposes only with great difficulty. The terms "humus" and "humic substances" are often used to designate the dark colored materials formed from plant and animal tissues, after these have become decomposed (the exact nature of the process of decomposition remaining practically unknown) and incorporated into the soil.

<sup>1</sup> Paper No. 276 of the Journal Series, New Jersey Agricultural Experiment Station Department of Soil Chemistry and Bacteriology. This is the first of a series of five papers dealing with soil "humus."

This process is usually termed "humification" and the organic matter thus formed and having become a part of the soil is spoken of as "humified," in contradistinction to the "unhumified" portion of the original organic matter added to the soil. The soil "humus" is sometimes [König et al. (121), Gehring (81)] differentiated as total "humus," obtained by multiplying the carbon content of the soil by an arbitrary figure (1.75), and available "humus," obtained by the action of some reagent, such as potassium permanganate or concentrated hydrogen peroxide, upon the soil organic matter.

Some investigators (largely American workers) apply the term "humus" to that part of the soil organic matter which is soluble in alkalis. Still others limit the use of this term to that part of the alkaline extract which is precipitated by acids [Page (168), Beckley (22)].

A similar variety of usages applies to the term "humic acid." Some [Loughridge (136)] consider "humus" to be present in the soil as a compound of "humic acid" and lime. When the latter is adsorbed by lime-loving plants, such as legumes, the "humus" is left in the soil as "humic acid." "Humic acid" is often considered as that part of "humus" which is extracted by alkalis. In some instances the term is limited to that part of the alkaline solution, which is precipitated by acids. S. Oden (162) designated by the term "humic acid" that part of the acid precipitate which is insoluble in alcohol, the soluble portion being the "hymetomelanic acid" of Hoppe-Seyler (106). A number of "humic acids" have thus been separated, largely according to their solubility in different reagents, and a number of formulae have been suggested for various "humus" and "humic acid" preparations (154, 98). Few of these are based on facts obtained by experiments and in the very few instances where claim could be laid to the existence of more or less definite chemical compounds in the soil (162), no attempt was made to learn just how these "humic acids" have originated in the soil and how they can be further decomposed. No attempt has ever been made to learn whether the "humus" of peat soils is the same as that of normal cultivated soils, although this has tacitly been assumed.

When fresh organic matter, in the form of plant or animal materials, is added to normal soil, some of the constituents of this organic matter begin to undergo rapid decomposition, in which a large number of microorganisms constantly present in the soil participate. The nature of these organisms depends on the nature of the soil (mechanical and chemical composition, physical condition) and on environmental conditions (moisture content, reaction and aeration, and presence of available minerals). The decomposition processes can best be followed by measuring one of the final products of the reaction, such as the evolution of carbon dioxide. The rapidity of decomposition depends on the nature of the organic matter, on the organisms active in the process, and on soil environmental conditions. After the rate of decomposition has reached a maximum, it begins to diminish until it reaches a certain condition of "equilibrium," if the soil is kept under the same experimental conditions. The carbon dioxide liberated accounts for only a part of the carbon of the

organic matter added to the soil. Another part will persist in the soil and will tend to become an integral constituent of the soil organic matter. The condition of "equilibrium" becomes established only when the readily decomposable constituents of the natural organic matter (sugars, starches, pectins, celluloses, proteins, amino acids) added to the soil have disappeared and only those constituents which are less readily acted upon are left. These and certain synthesized substances, both of which decompose only slowly, contribute to the soil organic matter. Once the early phases of decomposition, equivalent to the so-called processes of "decay" and "fermentation" have passed, the residual and synthesized organic matter and the organic matter of the soil itself (the soil "humus") undergo only a slow transformation, with the result that a moderate but constant stream of  $\text{CO}_2$  is being liberated and probably also, in a parallel manner, a constant stream of ammonia, which is changed, under favorable conditions, to nitrate (239).

Schreiner and Shorey (204) calculated that the average content of organic matter in the soils of the United States is 2.06 per cent and of the subsoils 0.83 per cent. This organic matter contains the essential element nitrogen and some of the minerals (phosphorus and sometimes potassium), which must be liberated in an available form, before they can be utilized again by cultivated plants. This can take place only when the soil organic matter is decomposed by the soil microorganisms. The decomposition is very slow, as can be readily demonstrated by placing a quantity of soil under favorable environmental conditions and measuring the rate of decomposition either by the evolution of carbon dioxide or by the accumulation of ammonia and nitrate nitrogen. Since the ratio between the carbon and nitrogen content of the organic matter in normal cultivated soils is more or less constant, approaching 10 to 1 (77, 211, 38, 236), the evolution of  $\text{CO}_2$  will be accompanied by a liberation of available nitrogen. The decomposition of the organic matter that has become a part of the soil and that will be referred to as "soil organic matter" is comparatively very slow because only limited and slow growing groups of microorganisms are capable of attacking it, as will be shown later. The decomposition processes can be greatly hastened in nonacid peat soils by draining, in acid peat soils by draining and liming, and in acid soils by liming. These treatments favor the development of the particular organisms which are capable of decomposing the "soil organic matter" and liberate the elements necessary for the nutrition of higher plants.

A knowledge of the formation, nature, and decomposition of the soil organic matter is the most important and most outstanding need of soil science today.

The earlier investigators assumed that soil organic matter is formed from plant and animal substances by chemical agencies, especially by the action of atmospheric oxygen and of water. Natural organic substances added to the soil were known to contain considerable quantities of various sugars and higher carbohydrates, proteins, and other nitrogen compounds, which are readily acted upon by microorganisms in the presence of sufficient moisture and at a

favorable temperature; it was also known that the soil harbors numerous microorganisms capable of bringing about the decomposition of those substances. Upon this knowledge was based the assumption that the soil microorganisms take an active part in the transformation or "humification" of the organic matter added to the soil (103, 102), but no experimental evidence was offered to throw light upon the various processes involved. The accumulation and the persistence of considerable quantities of organic matter in the soil without further rapid decomposition indicate that, although the natural organic matter contains substances readily acted upon by microorganisms, the organic matter that is formed in the soil is not very readily acted upon. This can be due to one or more of the following phenomena:

(a) the natural organic matter contains some substances which are readily decomposed and some which resist decomposition; (b) substances are formed from certain ingredients of the organic matter added to the soil which resist further decomposition; (c) the activities of microorganisms bring about a synthesis of substances which persist in the soil.

The chemist treated the soil with various reagents, usually acids and alkalies, alcohols, ether, pyridine, and other solvents and succeeded in isolating various complex bodies from the soil organic matter, but he gained only a limited amount of information concerning the origin of these bodies in the soil and their relation to the other organic and inorganic compounds. He has often compared the soil organic matter, or the so-called "natural humus" obtained by treating the soil with alkalies, with the dark colored substances produced artificially in the laboratory by the action of mineral acids upon carbohydrates ("artificial humus") and found in most instances no seeming differences between the two, forgetting entirely the fact that the soil harbors millions of organisms which are capable of breaking down sugar completely within a very short time and that mineral acids as such are absent in normal soils. The chemist was often led to conclude that the substances found in the soil are largely a result of the method of their extraction and in some instances no difference was even found between the "humus" of the soil and that of the natural organic materials added (86). The physicist and the physical chemist were interested in the soil organic matter largely as a physico-chemical colloidal complex; considerable information was thus contributed to the physical properties of this complex, especially in regard to the processes of combination of this complex with various bases ("adsorption" phenomena) but very little was contributed to our knowledge of the origin and nature of the complex (167). The microbiologist recognized early the numerous processes involved in the transformation of organic matter in the soil, the numerous organisms taking part, and the various complex reactions brought about, but he limited himself to gaining first information on the transformation of the simple ingredients of the natural organic matter, under controlled conditions, by pure or mixed cultures of microorganisms, without attempting to solve the more complex problems. The agronomist recognized the important rôle that soil organic

matter plays in soil fertility processes, but without knowing its nature and the conditions of its formation and accumulation he had no means of controlling its quantity and quality. That there is a difference in the quality of the soil organic matter and in the manner in which it decomposes is readily recognized when two different soils containing the same amounts of organic matter are compared; when the evolution of carbon dioxide or the accumulation of ammonia and nitrate nitrogen is used as an index, distinctly different results between the two soils may be obtained. Falck (66) clearly demonstrated that the nature of transformation of organic matter in forest soils and the type of soil resulting depend entirely on the organisms taking part in the decomposition and upon the environmental conditions influencing these processes.

The lack of sufficient knowledge concerning this problem which has attracted the attention of numerous investigators, including some of the most brilliant chemists, is due to its great complexity and to the faulty methods of investigations. The various constituents of the plant and animal tissues introduced into the soil, including fats and waxes, simple and complex carbohydrates, lignins, glucosides, proteins and their derivatives, alkaloids, pigments, tannins, phenol derivatives, and resins, are decomposed partly or completely, yielding a complex mass of dark colored substances, in which the identity of most original materials is lost. Instead of studying the genesis of "humus," attempts were made to determine its chemical nature. Various compounds have been isolated (204) from this complex mass, by different chemical manipulations; but the possibility has not been excluded that some of these at least were not present in the same form in the soil organic matter itself, but have been split off in the process of preparation. Nearly all the investigations dealing with the nature of the soil organic matter were limited to the soil itself, already formed, and few attempts have been made to study the course of its formation. As a recent investigator (231) expressed it, the need has been felt in

1. studying conditions under which the process of "humus" formation proceeds in one direction or another; 2. establishing what organic constituents of the plant vegetation are the sources of soil "humus;" 3. determining the agents of "humus" formation; 4. studying the chemical processes whereby the natural organic materials change into "humus;" 5. determining the chemical nature of the "humus" itself.

A complete review of the earlier literature on the soil organic matter, especially in reference to the "humic acids" is given by Wollny (250), Baumann (18), Grafe (89), Löhnis (134), Trussov (231), Czapek (45), Ehrenberg (59) and Oden (162). Attention is directed here only to those contributions which materially advanced the understanding of the nature of the soil organic matter and of processes leading to its formation.



## HISTORICAL

*The nature of soil organic matter*

Achard (2) in 1786 was the first to use alkalis as solvents for the extraction of a brown substance from the soil. A similar substance was extracted by Vauquelin (234) from an elm tree (*Ulmus*) infected with fungi. This substance was found to precipitate when the alkaline solution was acidified; it was partly soluble in alkalis and could form compounds with bases. The term "ulmin" was applied to this substance by Klapproth (174). De Saussure (194) was the first to express the idea that soil "humus" originates from vegetable matter by "the combined action of air and water." A number of contributions on the subject of soil organic matter soon followed, including those of Einhof (63), Braconnot (35), Proust (174), Berzelius (31), in which these substances were referred to under the terms "ulmin" and "humin." Braconnot (37) extracted from the rotted material collected at the root cavities of an old tree a substance soluble in alkalis and precipitated by acids, in the form of brown-black flakes, which he believed to be identical with "ulmin." *He found "ulmin" not only in the rotted organic matter but also in great abundance in peat and even in lignite.* He stated that ulmin must doubtless form an important constituent of "terre d'ombre," but it could not be obtained from coal. Braconnot was the first to obtain artificial ulmin by treating various organic substances with mineral acids, and lignous material with potassium hydroxide. Boullay (34) suggested that many vegetable materials are changed into ulmin under various influences; he found it in soil rich in vegetable matter, in manure, and in great abundance in peat soils. Ulmin, because of its ready combination with alkalis and its precipitation by acids, was looked upon as the best fertilizer.

The term "humic acid" was first suggested by Dobereiner (51) and then used by Sprengel (217), for the organic substances extracted by alkalis from soils. Sprengel, in 1826, was the first to describe the preparation, nature, and properties of "humic acid" and of its salts. He distinguished between "mild humus" and "acid humus" or peat, the latter originating in places where bases are lacking. It is interesting to note that this work carried out just a century ago exceeds in the understanding of the subject, in the accuracy of manipulations, and in the differentiation of the soil organic constituents, many of the contributions to the same subject published within the last few years and the treatment accorded the subject in recently published texts. According to Sprengel (217), "humic acid" is formed in the "fermentation" and "decay" of plants, whereby the larger part of its carbon combines with atmospheric oxygen and water. Sprengel, and Braconnot (36) previously, obtained artificial "humic acid" by treating woody tissues and plant residues with potassium hydroxide solution, the oxidation of the product by atmospheric oxygen being considered an essential part of the process. Sprengel stated that in a soil poor in bases, free "humic acid" remains without decomposing further, giving to the soil its acid reaction, as in the case of peat soils; when the soil is rich in bases, humic acid is further decomposed to carbon dioxide and water. Sprengel prepared humic acid by extracting pulverized air-dry peat with dilute hydrochloric acid for 2 hours, in order to separate the bases from the humic acid, then washing the residue upon a filter with water; the peat was then extracted with ammonia water in a closed vessel and the dark brown solution was neutralized with hydrochloric acid, which resulted in a precipitate. This precipitate was found to contain ferric hydroxide and clay. It was redissolved in sodium carbonate and reprecipitated in the cold with hydrochloric acid. "Humic acid" thus prepared was found to contain only traces of ash; it was shining black, easily pulverized, and possessed a great absorptive power for water. When freed from hydrochloric acid, the moist humic acid dissolved to a limited extent in water, but when dried, it became insoluble in water. On electrolysis, it went to the positive pole, behaving like an acid. It formed insoluble compounds (humates) with salts of alkali earths and with heavy metals. It combined with bases liberating  $\text{CO}_2$  from carbonates. Calcium and magnesium

humates were readily decomposed. The "humic acid" formed compounds with clay and with iron; it also liberated free silicic acid from soluble silicates.

The study of the subject so well begun was not advanced further during the following decades; as a matter of fact the introduction of new names and various formulae only tended to confuse it.

Berzelius (31) distinguished the alkaline-soluble fraction of the soil organic matter or "humic acid" from the alkaline-insoluble portion or "humin." Malaguti (141) demonstrated that, as a result of the action of hydrochloric and sulfuric acids upon sugars, "humic acid" and "humin" are formed. The product contained 57.5 per cent carbon and was considered to be very similar to the natural "humic acid" studied by Sprengel. All acids, both organic and inorganic, even in very dilute solutions, were found to give, on warming with sugar, "humic acid." The subject was further extended by Berzelius' pupil Mulder (152, 153, 154) who added new names and attempted to suggest chemical formulae for brown and black organic substances, found in decomposing organic matter and in artificial preparations. Mulder's name is usually quoted more often in connection with these organic soil compounds than that of any other investigator, largely because, as pointed out by Baumann (18), he has not given credit to Sprengel in his well known text, where he has placed in the background Sprengel's work, in comparing it with his own. The various terms and designations proposed by Berzelius and Mulder for substances, whose very existence is doubtful—crenic acid, apocrenic acid, geic acid—have appeared since in the literature and have persisted until the present time. Mulder dried his preparations at 140°–195°C., where he no doubt obtained considerable decomposition. He suggested at first that the nitrogen is present in the "humic acids" as ammonia, but later he declared that it must be present there as a sort of protein. Both Berzelius and Mulder considered "humic acid" to be present in the soil not as a free acid, while "humus" substances in general were not considered as acids, but were believed to become acids as a result of the alkali treatment.

Hermann (98) separated the various "humus" compounds into those that are: 1. soluble in alkalies and precipitated by mineral acids; 2. soluble in alkalies and not precipitated by mineral acids, 3. insoluble compounds. Hermann, like Mulder, believed that humus compounds can absorb nitrogen from the atmosphere. The nitrogen content of the "humic" bodies varied according to the formulae proposed, as  $C_{32}H_{32}O_{14}N_2$  (4.5 per cent nitrogen) for "indifferent humus bodies," and  $C_{12}H_8O_4N_2$  (11.6 per cent nitrogen) for "peat iso-crenic acid" or acid precipitated not by mineral acids but by metallic salts in solution acidified with acetic acid. The names and formulae suggested by Berzelius, Mulder, and Hermann can hardly stand criticism, but these authors have definitely pointed out that the soil organic matter consists of a number of substances, some of which contain nitrogen and some of which are free from it. The system of separating these substances, based on the solution by alkalies and precipitation by acids, proposed by these investigators is still the best that is available at the present time.

Detmer (50) came to just the opposite conclusion, namely, that the substances, which are dissolved by alkalies from peat, from soil, or from "humus" prepared artificially from sugar, and which are precipitated by an acid from the alkaline solution are all made up of a single compound—"humic acid" of the formulae  $C_{40}H_{54}O_{27}$ . The nitrogen was believed to be present in this body in organic combination and was considered as an impurity. The only substance different from "humic acid" was "humin acid," which was soluble in water. By repeated solution in potassium carbonate and finally in water and by reprecipitation with boiling hydrochloric acid, the nitrogen content was reduced to a minimum (from 1 or 2 per cent to 0.179 per cent). The "humic acid" thus obtained was similar in its properties to the natural product, although less soluble. Detmer's results seemed to point to the fact that the "humic acids" prepared from peat soils, by extraction with alkalies and precipitation with acids, consist of a nitrogen-poor or nitrogen-free compound and a substance rich in nitrogen. The nitrogen content of the "ulmin" from brown peat was found to be 0.64 per cent, of

"humin" from black peat 1.01 per cent, of "humic acids" from garden or field soils 3.3 to 3.6 per cent.

Eggertz (57), however, considered nitrogen to be a constituent of the "humic acid" molecule; he believed that iron and sulfur (0.5 to 2 per cent) belong also to the molecular complex. When "humic acid" was determined by Grandeau's (90, 91) method; namely, by washing soil with hydrochloric acid, treating with ammonia, and precipitating the ammoniacal extract with a mineral acid, phosphoric acid was always found in the extract. Eggertz, therefore, concluded that all constituents of the living cells form a part of the "humic acid" molecule. The natural "humic acids" could thus not be considered as definite chemical compounds of carbon, containing hydrogen and oxygen in the ratio of water; they could not be compared with the artificial substances prepared by boiling carbohydrates with acids and alkalis, for the latter lack both the nitrogen and the ash. The difference between the natural and artificial products has also been established by other investigators. Miklausz (147) found that natural "humic acids" contain less carbon than the artificial forms. According to Robertson, Irvine, and Dobson (184), the natural compound contains 1.71 to 2.47 per cent of methoxyl groups, whereas the artificial forms were found to contain 6.47 per cent methoxyl. According to Sestini (206), the natural "humic acids" also contain considerably more furfural than the artificial forms.

The nature of the artificial "humic acids" prepared from carbohydrates has attracted the attention of a number of investigators; it is sufficient to say, however, that these preparations were found to vary greatly in composition depending on whether they were prepared by the action of acids or alkalis, on the nature and concentration of the acid (dilute acids giving alkali-soluble "humin acids" and concentrated acids giving alkali-insoluble "humins") or alkali, and on the nature and concentration of the alkali used for extraction of the preparation. As these involve complex chemical reactions, which depend upon a number of factors, Baumann (18,19) came to the conclusion that the chemical formulae suggested for artificial humic acids deserve as little consideration as the formulae suggested for the natural humic acids. One can understand, therefore, how little basis there is for comparison between the natural and artificial forms of "humus." The work of Sestini (206) and Fröh (80) on the morphology of the "humus" compounds tended to confirm the observation of Mulder that these substances change to "humic acids" only on contact with alkalis.

Notwithstanding the various suggestions to the effect that "humic acids" are complex bodies, Berthelot and André (30) made an attempt to characterize both artificial and natural "humic acids" as definite organic acids. The brown, almost insoluble precipitate obtained by boiling sugar with hydrochloric acid was considered not as a mixture of different substances, but as an anhydride of "humic acid," which gradually changes, under the influence of water, into the hydrate. The "humic acids" were found to form, with sodium and potassium, monobasic salts soluble in water and tribasic salts insoluble but later decomposed in cold water. These investigators definitely noted that "humic acids" do not absorb nitrogen from the atmosphere.

The attention of the investigators was thus centered not on the soil organic matter as a whole but only on one certain definite part which was differentiated from the rest by its solubility in alkalis. Some, like Sprengel, Boullay, Malaguti, and Mulder, considered this "humic acid" to be a definite chemical substance. Sostegni (216) extracted soil with boiling sodium hydroxide, then precipitated the "humic acid" from the alkaline solution with hydrochloric acid; the precipitate was redissolved in alkali, reprecipitated with acid, and washed with water. The purified substance when treated with 85 per cent alcohol was separated into two fractions, one soluble in alcohol and the other insoluble. The soluble part contained 62.2 to 63.7 per cent carbon and the insoluble part contained 57.5 to 57.8 per cent carbon. Similar ideas were expressed by Hoppe-Seyler (106), who designated the alcohol-soluble portion as "hymetomelanic acid." Oden (162) separated the soil organic matter into 4 complexes, on the basis of solubility in alkalis, in water, and in alcohol:

1. The substances which are insoluble in dilute alkalies, but which are gradually dissolved by fusing with strong alkalies; namely, "humus coal," "ulmin" or "humin."

2. Substances soluble in alkalies, precipitated by acids, and insoluble in alcohol; namely, "humic acid."

3. Substances soluble in alkalies, precipitated by acids, and soluble in alcohol; namely, "hymetomelanic acid" and "ulmic acid."

4. Substances soluble in alkalies and in acids; namely "fulvic acid." Hoppe-Seyler and Oden are the outstanding later workers who claim a definite chemical composition for the various organic complexes in the soil.

Strache and Lant (219a) suggested that the different humic acids may consist of the same chemical compound, accompanied by various impurities; and that for the present it may be advisable not to attach any special names to the compounds obtained by different methods of procedure. At the same time they suggest separating the natural compounds, produced from the decomposition of organic materials ("natural humic acid" soluble in alkali carbonate and precipitated in acids, "humin" insoluble in alkali carbonate but soluble on continued boiling with hot alkalies, "humus coal" insoluble even on continued boiling with strong alkalies) from the artificial compounds obtained by treatment with chemical reagents ("artificial humic acid" or "huminoic acid" soluble in alkali carbonate and precipitated by mineral acids, and "artificial humin" or "huminoic substances" similar to "natural humins").

Sestini (206) demonstrated the presence in "humic acid" of anhydrides and ethereal substances, and of hydroxyl and alkyl groups, in the form of furan and benzol derivatives. Mik-lauz (147) found that when first the waxes are removed from peat by ether and alcohol extraction, then the bases by hydrochloric acid extraction, the treatment of the soil with alkali gives "humic acids" of greater solubility in alcohol than otherwise; the alcohol-insoluble part gives also a greater pyridine-soluble fraction than otherwise. Preliminary treatment of the soil with hydrochloric acid was found to result in an increase in the carbon content of "humic acids." The preliminary treatment of the soil with alcohol and acid resulted in a loss of 33 per cent of the organic matter. All alcohol-soluble substances were also found to be soluble in pyridine. Schreiner and Shorey (203) finally succeeded in separating from the "humic acid" a series of organic compounds some of which are colorless and some colored. Some of these are probably obtained as a result of the action of the reagents employed on the complex soil organic matter. The idea of the complexity of the soil organic matter including the "humic acids" is well expressed in the summary of the complex by Shmook (208): (a) Humic acid is a nitrogenous body of an acid nature, the acidity being due both to its power of adsorption, as a result of the colloidal condition of the humic acid, and to the presence of COOH groups. (b) Humic acid contains a tri-valent benzol ring. (c) A large part of the nitrogen in the "humic acid" is in the form of protein combined in a physico-chemical manner with the other part of the substance. A small part of this protein is less firmly combined and can be extracted by neutral salts; the protein of the humic acid is characterized by a high content of amide nitrogen. (d) The elementary composition of the humic acid is C—61.84 per cent, H—4.21 per cent, N—3.28 per cent, O—30.67 per cent.

The chemical composition of the so-called humic acids was thus found to depend largely upon the laboratory method of preparation; namely, the nature and concentration of alkali and acid used, and the temperature and length of action of the reagents. There is no wonder, therefore, that no comparison could be made between the results obtained by different investigators. The chemical formulae suggested as a result of data obtained by elementary analysis could not lay claim to accuracy, since no chemically pure substance could be obtained by the use of alkalies as solvents. In summarizing the previous investigations on the "humus" bodies of the soil, Schreiner and Shorey (203) noted that "the most conspicuous feature of this work is the discordant results obtained for bodies bearing the same name and often obtained from the same source."

But while some investigators were attempting to learn the nature of the soil organic matter or of certain of its constituents, others were attempting to compare the "humus" content of the soil with its fertility. The statement made by Hilgard (101, 102) that "unhumified" organic matter does not nitrify and that, therefore, it is unavailable for plant growth, led to various determinations of the "soil humus," "humus ash," and "humus nitrogen," without even any attempt to learn what these substances are and how they originated. In most instances, the original method of Grandeau (90) or one of its numerous modifications was used. It is sufficient to cite the work of Loughridge (137), Alway (7, 9), Kelley and McGeorge (116), and C. B. Lipman (131). These studies were not very fruitful in consequences, and the method of making "humus" determinations is all but abandoned, at present. It is of interest to note here that Hilgard found that the narrower the carbon-nitrogen ratio of soil "humus", the less is the nitrogen need for the growth of higher plants.

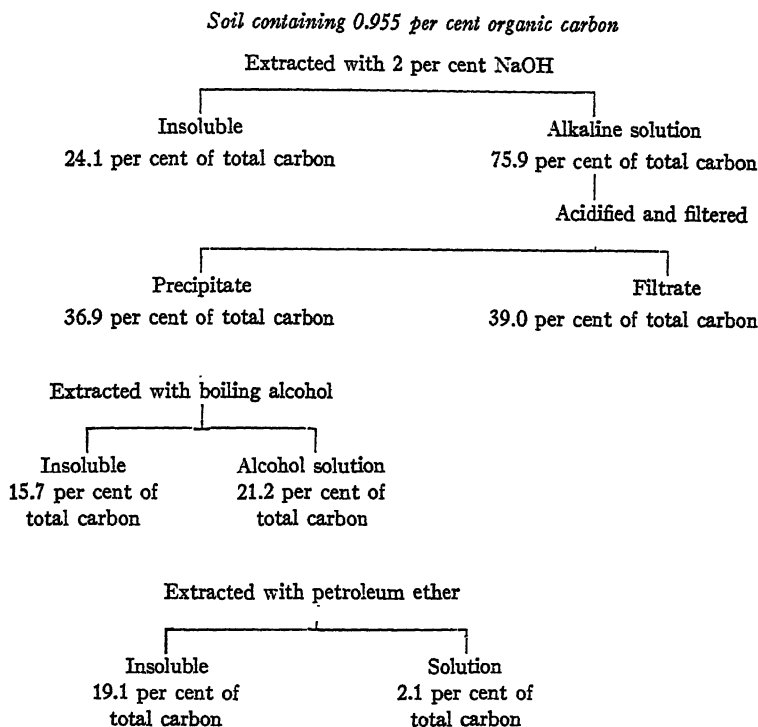
Several contributions to the subject of composition of the soil organic matter, made within recent years, however, have helped to obtain a better insight into the nature and origin of soil "humus." The work of Schreiner and Shorey (203, 204) and their collaborators of the Bureau of Soils is of especial importance in this connection. These investigators were the first to demonstrate definitely that the soil organic matter is not a single compound or a group of chemical compounds, but is made up of a large number of compounds. That the numerous plant constituents are in some mysterious way conglomerated into a single group of closely related bodies, the "humic acids," as assumed by a number of chemists and agronomists, is entirely disproved. Not only is the soil organic matter made up of a large number of compounds, but these are not closely related and belong to different classes, the types of compounds of soil organic matter being quite as varied as in plants or animals. Schreiner and Shorey have isolated from the acid filtrate, after the precipitate formed by the acidifying of the alkaline extract of soil has been removed, the following compounds: dihydroxystearic acid, picoline carboxylic acid, xanthin, hypoxanthin, cytosin, histidin, arginin, and pentosan; this mixture was usually classified as "crenic" and "apocrenic acid" and recently renamed by Oden as "fulvic acid." From the precipitate, usually referred to as "humic" or "ulmic" acid, they have isolated resin acids, resin esters, glycerides, paraffinic acid, lignoceric acid, agroceric acid, agrostol, and phytosterol.

Schreiner and Shorey (204) suggested the method shown on page 133 for the separation of the different soil fractions.

Their statement that "the material known as soil organic matter is in the transition stage from the complex compounds of living organisms to the simple ultimate products" can be partly questioned, but they rightfully suggested, in the case of a number of compounds, that the synthesizing activities of the soil microorganisms probably have to do with their presence in the soil.

Notwithstanding this work, Oden (162) still adheres to the ideas of the earlier chemists that the soil organic matter consists of a few definite chemical compounds, classified into 4 groups, as shown above. In this respect, no advance has been made over the work of Berthelot and André (30) who divided the soil organic matter on a similar basis, but who found nevertheless that the different fractions vary in the relative amount of carbon to nitrogen, as shown in the following summary:

ORGANIC MATTER FRACTION	FRACTION CONTAINS PER CENT OF TOTAL CARBON	DISTRIBUTION OF THE TOTAL NITROGEN IN FRACTIONS	CARBON NITROGEN RATIO
		<i>per cent</i>	
Part insoluble in cold alkalis. . . . .	31.7	1.25	25
Part soluble in alkalis and precipitated by acid. . . . .	27.6	1.52	18
Part soluble in alkalis, but not pre- cipitated by acids. . . . .	40.7	3.9	10.3



The fraction resisting the action of alkalis thus contains the least nitrogen, whereas the fraction that was made soluble by the alkali treatment and that cannot be precipitated by acids, contains most of the nitrogen. The composition of the "humic acid" or that part of the "humus" which is soluble in alkalis, but insoluble in acids, was as follows: C—55.2, H—6.8, N—3.9, O—34.1.

"Humic acid" was considered by Oden (161, 162) as a definite chemical complex. In differentiating between "humic compounds" and "humic acids," Oden defined the first as "the light-brown to dark-brown substances of unknown constitution which are formed in nature by the decomposition of organic matter through the agency of atmospheric agencies or in the laboratory by chemical reagents," and the second as "those humic substances which are capable of giving hydrogen ions and form typical salts with strong bases." The nitrogen was considered as an impurity. No attempt was made at a systematic study of the origin of the soil organic matter, which would throw light upon the nature of these so-called "humic acids."

Just as did the earlier investigators, Oden isolated his "humic acid" from peat soils, by treating them with acid, washing with water, and extracting with four-normal alkali solution. The alkali treatment was repeated 15 to 20 times. The solution was then treated with sufficient NaCl to make two-normal. A coagulum was formed and filtered off; the filtrate was then concentrated and acidified with hydrochloric acid. The precipitate contained both the so-called "humic" and "hymetomelanic" acids, which were separated by alcohol extraction. By means of the potentiometer, Oden demonstrated that the "humic acid" ion is actually present in the soil; he suggested, therefore, that the acid nature of the soil organic matter is due to actual hydrogen ions formed by "humic acid." By determining the hydrogen-ion concentration of this "humic acid" prepared from peat, Oden obtained a reaction of pH 3.87,

but the reaction of natural peat materials was found to range from pH 3.13 to 4.09. Gillespie and Wise (82) found that the hydrogen-ion exponent of "humus," after the first preliminary washings, was pH 4.15; when normal KCl solution was added for washing, the acidity increased to pH 3.3, but on washing further with HCl solution, the exponent increased to pH 4.65. The existence of a soil acidity of pH 4.0 to 4.5 could, therefore, be explained by assuming the actual liberation of free hydrogen ions or the existence of a "humic acid." How could a greater acidity, which can readily be demonstrated in certain peat and other soils, be explained? Oden recognized this fact and suggested that the acidity of acid soils is caused by other substances than those classified as "humic acids." Oden's theory—that "humic acid" is a tetra-basic acid, of a definite chemical composition and free from nitrogen—could also not account for the nitrogen which is usually present in these "acids," in amounts ranging from 2.5 to 3.5 per cent. On repeated solution and reprecipitation, the nitrogen content of the preparation was reduced from 2.5 to 0.7 per cent, thus confirming the results of Detmer (50), who claimed that the nitrogen is an impurity of the "humic acid." This lower nitrogen content could not correspond, however, to the "humic acid" with four replaceable hydrogens. Oden, therefore, also concluded that the nitrogen was probably an impurity. To explain the existence of specific "humic acids," Oden had to suggest that the soil acidity is due not to its hydrogen ions only, and that the nitrogen always found in definite amounts in these "humic acids" is an extraneous constituent.

Van Bemmelen (25) and Baumann (18) give a different interpretation of the nature of the soil organic matter. According to van Bemmelen (26), "humic acids" are not organic acids; they are amorphous and colloidal in nature, having originated from plant substances by various chemical processes, including those of hydrolysis, dehydration, and oxidation, depending on the microorganisms active in the process and on environmental conditions; the amorphous complex was believed to consist largely of carbohydrate and protein decomposition products. On comparing the "humic acids" from peat soils with the extracts from the sphagnum plants which go to make up these soils, Baumann and Gully (19) also concluded that "humic acids" are not acids. The organic material of the soil was looked upon as a colloidal complex with a high power of adsorption. When a salt is added, the base is absorbed by the colloidal complex, setting the acid free; it is this acid which was thought to cause soil acidity. The electrical conductivity of these so-called acids was found to be much less than that of a 0.5 per cent solution of acetic acid and even that was thought to be due to a slight admixture of organic and inorganic acids as impurities.

The rôle of "humic acids" in producing soil acidity is thus explained: 1. by their absorption power, when considered as colloidal complexes (19); 2. by the dissociation of hydrogen ions when considered as organic acids (4, 5, 82, 161, 162) or 3. by the replacement of the inorganic acid, when treated with salts (47, 115).

This brief historical review of the nature of soil organic matter indicates our entire lack of definite systematic knowledge concerning this important soil constituent and the numerous conflicting opinions on the subject. It should be mentioned here, however, that even those investigators, like Oden, who believed that "humic," and other acids are definite chemical compounds, admitted that these are accompanied in the soil by a large number of other chemical substances, which may be classified as follows:

1. Various inorganic and organic acids, such as phosphoric, sulfuric, formic, acetic, propionic, malic, levulinic, oxalic, succinic, dihydroxystearic, and picoline-carboxylic.
2. Aldehydes (214), waxes and fats. Post (175) found that air-dry soil rich in organic matter contained 0.52 per cent fats and waxes; Reinitzer (178) found 0.184 per cent ether extractives in a forest soil, of which 0.154 per cent was wax and 0.03 per cent fat; Fraps and Rather (79) found an average of 0.0203 per cent ether extract and, in addition, 0.0174 per cent

chloroform extract, on an average of 28 Texas soils. According to Schneider and Shellenberg (199, 200), the quality of ether extractives in peat increases with the age of the peat. Dachnowski (46) reports as much as 4 per cent of ether-soluble substances found in certain peat preparations.

3. Various carbohydrates and alcohols, such as pectins, pentosans (68, 147, 206), phytosterol, agosterol, and cholesterol. Schreiner and Shorey (203) found that the pentosan carbon made up 1.30 to 28.5 per cent of the total carbon of the soil; V. Feilitzen and Tollens (68) even suggested the possibility of using the pentosan content of peat as a measure of the degree of its decomposition [see Gorbenko (83)]. According to Fraps (77), there is a more or less definite ratio between the pentosan and nitrogen content of the soil.

4. Nitrogenous substances, like xanthin, cytosin, histidin, arginin, leucin, isoleucin and other protein-split products, isolated by Schreiner and Shorey (203), Robinson (185, 186, 187), and others. According to Stutzer and Klingenberg (220) some of the nitrogen is present in the soil in a form which is only very slowly available and which consists of plant and animal nucleins, but no attempt has been made to learn whether these substances are present in the soil as such or in the form of more complex compounds. A large part, if not most, of the nitrogenous compounds reported to have been found in the soil are no doubt present as dead and partly decomposed bodies of microorganisms or certain plant residues. Schreiner and Shorey (203), for example, found that dihydroxy-stearic acid occurs in fungus mycelium. Chitin, which is a characteristic ingredient of the cells of various microorganisms has also been often found (155) in the soil, Post-Ramann (175) having recorded that 15 to 20 per cent of the dry weight of peat soils consists of chitin; Höveler (107) could not demonstrate, however, any chitin in peat soils. Potter and Snyder (172) found that the humin nitrogen, as determined by the Van Slyke method of protein hydrolysis, is very high in soils in comparison with the humin nitrogen of proteins; the amount of amino acid and peptide nitrogen found in the soil is very small as compared with the amount of amino acid obtained by acid hydrolysis of soil. Potter and Snyder (172) and Morrow (151) have shown further that the organic matter, as distributed by the Van Slyke method, is essentially the same in soils differently treated and in different soil types. In other words, the nature of the nitrogen distribution in the soil organic complexes is different from that of the nitrogen distribution in proteins of plant and animal origin, a fact also established by Lathrop (129); this nitrogen distribution also indicates a similar kind of nitrogen found in different soils.

#### *Quantitative methods of measuring "humus" in the soil*

Before considering the various theories explaining the origin of "humus" in the soil, it is of interest to review the methods commonly used for determining quantitatively the organic matter content of the soil. Some of these methods are based upon the determination of the total organic matter in the soil, whereas others measure only that part of the organic matter which is more readily oxidized; some determine the organic carbon, others measure, by different methods, the portions of the organic matter which is soluble in alkalies, whereas still others determine only the part of the alkaline extract which is precipitated by hydrochloric acid.

The most common method for determining the "humus" content of the soil has been that of Grandeau (90) and its various modifications (102, 177, 116). The method consists in extracting the soil first with a dilute solution (about 1 to 10 per cent) of hydrochloric acid, for various lengths of time (until free from calcium), then washing with water. This extraction is presumably for the purpose of washing out the soil bases, with which the "humus" or "humic acids" are otherwise combined. The washed soil is then extracted with ammonium



hydroxide and the clear extract evaporated to dryness, weighed, and ignited to determine the ash content. In other methods, the alkaline extract is precipitated with acid and the precipitate weighed.

It was found, however, that the concentration of the ammonia solution and the time of extraction are of importance; Huston and McBride (108) suggested, therefore, the use of a 4 per cent ammonia solution and a 12-hour extraction period. The ammonia was found to deflocculate the clay, which is not removed by filtration, giving varying results. Various suggestions were then made to remove the clay by filtration through a clay filter (39, 40, 116); to coagulate the clay by evaporation on a water bath (150) by the use of ammonium sulfate (218) or potassium chloride (78); or to separate the clay by electrolysis (177) or by centrifuging (243). None of these modifications, however, made the determinations of "humus" more reliable. The fact that different alkalis extract different amounts of "humus," and that the latter varies in composition, depending upon the nature of the extracting agent (84, 85), served further to discourage the laying of too much emphasis upon the quantitative determination of "humus." Alway, Files, and Pinckey (9) and Fraps (76, 77), recognizing that a large number of the past determinations of "humus" were unreliable, ascribed this to the high ash content.

The "humus" determined by the various methods represents a mixture of organic substances obtained as a result of a certain chemical operation; every modification in the method results in the modification of the nature of the substances extracted. Oden (162), working with peat soils, said, "These methods may be satisfactory for mineral soils, but not for peat soils rich in organic matter." However, the organic matter in mineral soils interacts partly with the inorganic soil constituents, especially aluminum salts; a part of the organic matter forms a complex with inorganic materials, which is soluble both in acids and alkalis, but is precipitated at a definite isoelectric point of pH 4.8 (237). As a result of this, the amount of organic matter extracted will depend on the nature of the reagent used.

Piettre (171) used pyridine for the extraction of "humus" from soil. The "humus" obtained after the solvent is evaporated, is extracted with ether and alcohol and is ashed. The residual soil may be dried, extracted with dilute HCl, washed and dried, then again extracted with pyridine. Balks (17) found that pyridine extracts only the "humified" organic matter, whereas the straw particles remain practically unattacked; but the results thus obtained are claimed to be unreliable.

Colorimetric methods (20, 166, 145, 56) have often been utilized for measuring the "humus" content of the soil or "the degree of humification" of the soil organic matter. A 10 to 15 per cent NaOH solution is used for the extraction of the "humus;" the color of the unknown extract is compared with that of a known solution of "humic acid." According to Oden (162) an increase in the concentration of alkali does not increase the "humification number." Gortner (84) reported, however, that the soil pigment is not necessarily the same substance as the soil organic matter. Alway and Pinckney (11) found that, for surface soils of the same locality, the intensity of color of the ammonia extract is closely concordant with the amount of "humus" present; but, on comparing semi-arid and humid subsoils, they obtained a brown color for the first and no color for the second, although both soils contained the same amount of "humus" when determined gravimetrically.

In many instances, the determination was limited to only one of the constituents of the alkaline extract of soils, such as the methoxyl group (146, 207), total carbon (84, 85, 246), total nitrogen (101, 112), amino or amide nitrogen (119, 52, 13, 14, 123, 223, 172). The results were, however, far from uniform. The discrepancies in the nitrogen determinations of

the soil "humus" are especially illustrative. Hilgard and Jaffa (103) and Loughridge (137) measured the nitrogen content of "humus" by subjecting to the Kjeldahl method the ammoniacal extract of soil, which was evaporated to dryness. A very high nitrogen content running up to 20 per cent was found. Instead of considering the possibility of a faulty method, since no natural organic substance could contain more nitrogen than the purest proteins, they concluded that this high nitrogen content is characteristic of arid soils. On the basis of these faulty determinations, they constructed a theory that although humid soils are rich in "humus" of a low nitrogen content, arid soils contain a smaller amount of "humus" but of a higher nitrogen content. Hilgard saw in this a tendency to equalization of the "total nitrifiable nitrogen content," as the "humus" nitrogen was referred to, due to the fact that it is this form of nitrogen that was supposed to be the most available in the soils of the two regions. This statement was accepted without any attempt at a critical review of the results by the subsequent text writers (247). C. B. Lipman (131, 132), pointed out, however, that the results of Hilgard were due to the faulty method used in extracting the soil "humus." When sodium hydroxide instead of ammonium hydroxide was used for extraction, no difference was found in the nitrogen content of the "humus" of arid and humid soils. This was further substantiated by the results of Sievers and Holz (212), who found that the carbon-nitrogen ratio of soils, irrespective of their origin, is practically constant. A detailed review of the various methods for the determination of "humic acid" in the soil is given by Hoering (104) and Oden (162). White and Holben (246) found that the carbon-nitrogen ratio of the "humus" obtained by washing soil (20 gm.) with 3 per cent NaOH on a Buchner funnel (150 cc. collected) is the same as that of the soil itself. By making the extraction in a shaking machine, the amount of "humus" obtained increased with the length of extraction; shaking for 88 hours with 3 per cent NaOH gave 56.8 per cent of the "humus," whereas extracting for 3 hours on a Buchner funnel gave only 30.5 per cent. By comparing the amount of "humus" in the soil with the crop yield, for a series of differently treated plots, they came to the conclusion that the determination of soil humus, by multiplying the total organic carbon of the soil by 1.724, is a more reliable index of soil productivity than the determination of "humus" by extraction with alkalis.

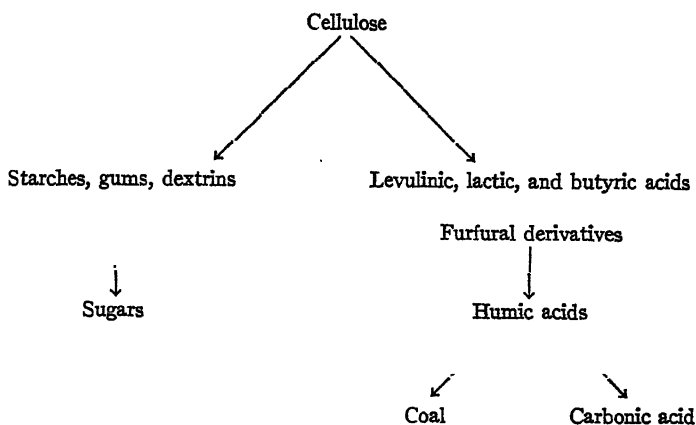
Various other methods are commonly employed for determining the quantity of the total or "available" soil organic matter:

1. The liberation of  $\text{CO}_2$  from carbonates (finely divided  $\text{CaCO}_3$ ) by the free "humic acids" (224). This method, which was later modified by Stüchting (221), consisted in treating the "humus" with a definite amount of  $\text{Ba}(\text{OH})_2$  solution, then adding an excess of  $\text{BaCl}_2$  and titrating back the excess of hydroxide with standard acid.
2. The amount of iodine liberated by an equivalent portion of "humic acid" from iodates in the presence of potassium iodide (19).
3. The color of the aqueous solution of "lithium humate" formed by the action of "humic acid" upon  $\text{Li}_2\text{PO}_4$  (4, 5).
4. Titration of soil "humus" with  $\text{CaCl}_2$  solution. The method was found (33) to yield only unreliable results.
5. Oxidation of soil organic matter with  $\text{KMnO}_4$ , followed by determination of the organic carbonate going into solution (16, 109). Fallot (67) found that 8 gm. of oxygen used (as  $\text{KMnO}_4$ ) is equivalent to 3 gm. of carbon or 6 gm. of organic matter.
6. Treatment of 1 or 2 gm. of soil with 60 cc. of 6 per cent  $\text{H}_2\text{O}_2$  solution for 15 minutes, filtering, washing with hot water and determining the loss of organic matter by ignition (121, 188). It is interesting to note here that König and Rump (122) found that lignins could be removed from wood by oxidation with  $\text{H}_2\text{O}_2$ .
7. Oxidation with silver chromate, then multiplying the carbon content by 1.742 to give the quantity of "humus" (17).

*Origin of soil organic matter ("humus" and "humic acids")*

The earlier investigators studying the nature of soil "humus" and the various "humic acids" concerned themselves very little with its origin. Some vague statements that "humus" is a result of decomposition of natural organic substances of plant and animal origin by the action of atmospheric agencies or by microorganisms was sufficient. Baumann (18) and others considered "humic acids" to be a mixture of undecomposed plant tissues, such as the cell membranes of the hyaline sphagnum cells (in the case of peat soils), and partly decomposed substances of plant and animal origin, in the form of a colloidal complex. Hilgard (102) regarded soil "humus" as a definite soil product formed from vegetable material in the soil, under the influence of fungi and bacteria under aerobic conditions; he believed that nitrogen in plant residue must first pass through the "humus" stage before it can be nitrified and become available for the growth of higher plants. An extract similar to "humus" was obtained (78, 86) from fresh plant materials and it was found that when these materials are added to the soil, an actual decrease in "humus" takes place. The idea was then suggested that "humus" is not formed in the soil but is added to it and actually diminishes in the soil. These considerations seem contrary to our ideas of the resistance of soil "humus" to decomposition and of its accumulation in the soil. Some investigators (204, 111, 12) considered "humus" to be a complex of various substances formed from the decomposition of carbohydrates and proteins added to the soil.

Some of the earlier workers, like Detmer (51) and Czapek (45), considered celluloses as the mother substances of "humus," which was looked upon as some intermediary substance between the celluloses and the final product of decomposition, namely carbon dioxide. Among the more recent workers Bergius (28), Jones and Wheeler (114a), Chardet (42) and Marcusson (142, 143) are also of the opinion that celluloses give rise to "humus" or to "humic acids" and to coal. Bacteria are believed to change celluloses into oxycellulose, which is then changed into "humal" acid, then into "humic" acids. The fact that some peats contain a considerable amount of oxycellulose and that the organic complex obtained by the use of a dilute alkaline solution is different from that obtained by the use of a more concentrated solution, also that dark colored substances originate by warming furfural, which may originate from cellulose, was considered as sufficient evidence for this theory. Bottomley (34) and a number of other investigators claim that natural and artificial "humus" are the same. Chardet (42) believed that, under aerobic conditions, the celluloses are decomposed by bacteria into dextrins and sugars, and these into fatty acids and  $\text{CO}_2$ ; and that under anaerobic conditions, as in peat soils, oxidation processes do not take place and "humic acids" may be formed by the processes of condensation, according to the scheme on following page.



Hoppe-Seyler (106) was the first to demonstrate that celluloses and hemicelluloses do not contribute to the formation of "humic acids." He called attention to the rôle that lignin plays in this process. The more or less resistant xylans also remain undecomposed for a considerable time; they are soluble in alkalis and are precipitated by acids, thus forming a part of the "humic complexes." Unfortunately, Hoppe-Seyler carried out his studies with paper immersed in liquid media and inoculated with anaerobic organisms, whereby the growth of fungi and actinomyces was eliminated. Ehrenberg (58) also expressed doubts as to cellulose being the mother substance of soil "humus," since he could not obtain any "humus" in the decomposition of cellulose or its derivatives at normal soil temperatures. Snyder (215) was the first to study "humus" formation under conditions which would correspond to normal soils. "Humus" was formed at the expense of sugar, oat straw, clover hay, etc.; no attempt was made, however, to determine whether this "humus" originated from the organic materials themselves or was merely a result of the development of fungi. Trusov (231) actually demonstrated that there is no direct formation of "humus" from celluloses; indirectly these substances may have an important bearing upon the process, through the synthesis of microbial cell substance. This is true of course of other carbohydrates. Various other investigators (164, 223, 27) did not consider celluloses as "humus"-forming materials. According to Benni (27), the proteins of plant and animal origin, certain carbohydrates (not celluloses), and a few plant acids contribute to the process of "humus" formation: the oxidation of the proteins and their derivatives is supposed to give a nitrogen-containing "humic acid" and the oxidation of the carbohydrates and organic acids yields a nitrogen-free "humic acid," a mixture of the two being "humus."

The positive rôle of proteins in the formation of "humic acids" has been suggested by the work of various investigators; this process which takes place in the soil has been frequently compared to the formation of the dark colored melanins on boiling proteins with acids or alkalis. The subject has resulted in

an extensive literature (43, 193). However, Mulder (154) and Schmiedeberg (198) emphasized the fact that no two "humus" bodies ever obtained agreed in their composition. The formation of the dark colored substances in the boiling of proteins has been ascribed (128) to the carbohydrate present in the protein molecule or to certain specific amino acids, such as tryptophane or tyrosine (165, 87, 88). The benzol and pyrrol nuclei are largely concerned with the formation of "humus," which results from the activities of micro-organisms. It is sufficient to point to the formation of homogentisic acid from tyrosin by the action of actinomyces. The same is true of other products of protein decomposition, such as p-cresol, hydro-p-cumaric acid, and other phenol derivatives.

The protein nature of soil "humus" was first suggested by G. Fischer (72). Suzuki (223) and Jodidi (111) came to the conclusion that "humic acids" contain an insoluble body, in the nature of a protein, which contains the nitrogen of the organic matter. In this they confirmed the previous observations of Berthelot and André (29, 30), Sestini (207), Dojarenko (52), and Baumann (18), who found the soil nitrogen to be in the form of amino- and amide compounds, and of Sievers (213) and Grouven (93), who considered the nitrogen to be present in the soil "humus" in the form of a protein. Detmer (50) was the first, however, to demonstrate that nitrogen exists in the soil in the form of organic compounds. But Detmer himself and later Sestini (207), Oden (162), and Eller (64) were led to believe that the nitrogen is only an impurity of the "humus" or "humic acids" found in the soil, since on boiling these with hydrochloric acid, the nitrogen can be reduced from the 2.5-3.5 per cent to 0.7-1.5 per cent. One might be more justified in concluding that boiling soil organic matter with hydrochloric acid hydrolyzes the nitrogenous part of this organic complex more readily than the non-nitrogenous portion. Eggertz (57) found that, on reprecipitation, "humus" may become richer in nitrogen.

One may dispose easily of the earlier ideas of Mulder (154), Ritthausen (183) and others that the "humic acids" absorb the nitrogen only in the form of ammonia. The nitrogen found in the soil organic matter may be looked upon as either forming an integral part of the soil "humus" or as being present there in the form of proteins, as suggested by Berthelot and André (29), Eggertz (57), Shmook (208) and others. These proteins probably form definite complexes with the other constituents of the soil "humus." Whichever theory is accepted as the correct one, one fact is certain, that the nitrogen of the soil organic matter is derived either from the nitrogenous constituents of the plant materials added to the soil or from the constituents of the various microbial bodies bringing about the decomposition of these materials. It is a well known fact that when proteins and their derivatives are added to the soil, they are rapidly acted upon by the different microorganisms, with the formation of ammonia and other protein derivatives. If these were the forms of nitrogen present in the soil "humus," they could easily be isolated from the

soil without having to subject it to the vigorous treatment with strong acids. Only a part of the nitrogen can ever be found in this form, as shown by numerous investigators (129). Süchting and associates (222) suggested that the major part of the nitrogen is present in the soil in the form of heterocyclic compounds, such as derivatives of pyridine and its higher homologues, which cannot be decomposed readily by microorganisms; this nitrogen may be made available to plants, either in a normal condition by the interaction of mycorrhiza fungi, or when the soil is heavily limed and aerated. The actual isolation of a pyridine derivative—picoline carboxylic acid—from the soil was accomplished in 1906 by Shorey (209). Most of the studies on the nature of the nitrogen compounds of the soil, however, were limited to the demonstration of various amino acids and amides among the hydrolytic products obtained by the action of strong mineral acids upon the soil organic matter.

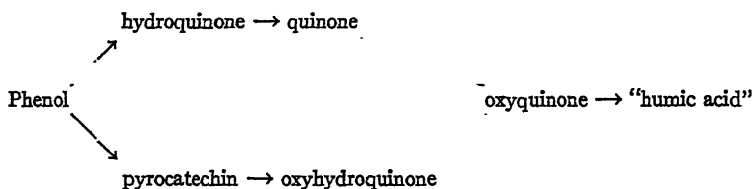
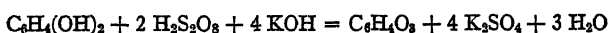
Lathrop (129) demonstrated that the decomposition of proteins in the soil is accompanied by various synthetic processes, including the formation of new proteins and nucleic acids which are more resistant to decomposition. The new protein is characterized by a much higher amide content than vegetable and animal proteins and points to the presence of considerable amounts of microbial protein in the soil.

Nägeli (157) was the first to ascribe to filamentous fungi the main rôle in the formation of crude "humus." Kostytscheff (124) demonstrated that "humus" is formed from various organic substances when acted upon by fungi and not by bacteria. Müller (155) considered *Cladosporium* as an important organism taking an active part in this process; Köning (120) ascribed an active rôle in humus formation to *Trichoderma köningi* and *Cephalosporium köningi*; Höveler (107) considered fungi as the most important agents in the process of "humification;" the results of Scherpe (196) point also in the same direction. According to Beijerinck (23), lignin is changed to "humus" chemically, whereas the epidermis and the dark tissues are acted upon by fungi. Beijerinck considered the actinomyces, because of their ability to form quinone from proteins formed in the cells of the organisms, to be active in the process of "humus" formation. The fact that fungus mycelium penetrates woody tissues and organic particles of forest and peat soils has been recorded by various investigators (180, 176). According to Frank (75), these fungi make up a considerable part of the soil organic matter and the nitrogen of the "humus" is largely derived from the cells of microorganisms. The formation of pigments by various bacteria (233) may play a part in the formation of the dark colored substances referred to as "humus." Beijerinck (24) and Rippel and Ludwig (181) found that the dark pigment of *Azotobacter chroococcum* is in the nature of melanin which is formed from tyrosin; it dissolves in sodium hydroxide solution.

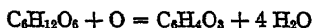
It seems to be generally agreed (134) that under anaerobic conditions, celluloses are completely decomposed to carbon dioxide, methane, and fatty acids, whereas, under aerobic conditions, various dark colored substances are left by

the bacteria and fungi. These substances are constituents of the microbial protoplasm and are not secretion products or intermediary products formed from the decomposition of natural organic substances. This has been demonstrated, in the case of fungi, by Daszewska (48), Traaen (230) and Waksman and Heukelekian (238); the cellulose is decomposed completely and all its carbon can be accounted for by the carbon dioxide of the atmosphere and the carbon assimilated by the microorganism. The only residual substances are the microbial cells. Our present information concerning the origin of "humus" or "humic acids," namely, the dark colored organic substances which resist further rapid decomposition can be summarized as follows:

1. *The formation of "humus" as a result of oxidation of benzene-ring compounds.* Hoppe-Seyler (106) and Reinitzer (178) demonstrated that dark colored substances ("humus") are formed on boiling phenols and quinones with alkalis. According to Eller (64), the oxidation of phenol, quinone and hydroquinone in an alkaline solution results in the formation of acids similar, not only in composition (58.05 per cent carbon) but also in the various physical and chemical reactions, to natural "humic acids," of the formula  $x(C_6H_4O_3)$



The natural "humic acid" obtained from brown coal with sodium hydroxide solution and precipitated with an acid was found to contain 59.6 to 60.2 per cent carbon, 3.2 to 3.4 per cent hydrogen, 1.7 to 2.0 per cent nitrogen, 1.2 to 1.9 per cent sulfur and 1.4 to 2.4 per cent ash. The nitrogen was looked upon as an impurity and not as a normal constituent of the "humic acid." Eller believed that natural "humic acids" originate by the oxidation of hexoses, as



He thus agreed with Bottomley (33), Mascusson (143), Jonas (114) and Jones and Wheeler, (114a) who ascribed the furan structure not only to the synthetic "humic acids" from carbohydrates, but also to the natural "humic acids." Muschel (156) found that the black coloration of the nutrient media of *Bac. mesentericus niger* was due to components of the benzene ring series, closely related to o- and p-dihydroxybenzene, which apparently form condensation products with amino acids. Similar results were obtained by Perrier (169), who found that when alkali salts of benzoic and salicylic acids and phenol are acted upon under aerobic conditions by fungi and bacteria (*Bact. pyocyanum*) a characteristic dark colored substance is formed. Perrier concluded that the dark colored substances of "humus" are formed by the oxidation, at an alkaline reaction, of cyclic compounds present in animal excreta and in vegetable substances, after they have decomposed. The formation of dark colored substances from tyrosin and other phenol derivatives by actinomycetes was referred to previously.

2. *The formation of "humus" is believed to be a result of the interaction of carbohydrates with amino acids or polypeptides.* Various investigators recognized that nitrogen is an essential part of soil "humus." André (13) claimed that this nitrogen is fixed so energetically to the carbon of the "humus" that when it is boiled with a solution of NaOH or KOH, only a part is

eliminated as  $\text{NH}_3$ ; only prolonged treatment will remove more of it. This confirmed the earlier observations of Eggertz (57). Maillard (140) considered that nitrogen plays a necessary part in determining the natural processes of humification.

As far back as 1861 Thénard (227) stated that "humic acid" found in manure is not derived from animal material but by the action of animal matter upon lignins or substances extractable from straw or wood. By extracting manure with warm water and precipitating the alkaline liquid with hydrochloric acid, Dehérain (50) obtained a product which analyzed 59.2 to 63.0 per cent carbon and 3.0 to 4.7 per cent nitrogen. Dehérain (49) as well as Hébert (94) observed that, in the rotting of manure, a constant transformation of the ammonia nitrogen into organic nitrogen takes place. Dehérain recognized in it the formation of microbial protoplasm. Maillard (139) believed this to be a purely chemical reaction of sugars and amino bodies. He believed that the rôle of microorganisms in this process consists in the breaking down of proteins to polypeptides and amino acids; the actual formation of "humus" is, according to Maillard, an automatic chemical reaction (between the amino acids and carbohydrates), in which microorganisms play no part. The natural and artificial humic substances (latter obtained by condensation of sugars with amino acids) were believed identical in composition, resistance, and nitrogen [see also Roxas (191)]. However, Neuberger and Kobel (158) could not obtain either melanin or  $\text{CO}_2$  formation as a result of interaction of fructose with alanin in the cold, even after weeks. Although dark colored bodies are formed in the interaction of amino acids with sugars at high temperatures and in concentrated solutions, it is doubtful whether this would take place to any extent in the soil (110, 231).

3. "*Humus*" is formed from the polymerisation of furfural. When furfural is boiled with hydrochloric acid, it is converted into a black insoluble mass (87, 88, 142). It was suggested, therefore, that "humins," which is formed when carbohydrates are boiled with acids, is actually formed from furfural, which is produced in its turn from carbohydrates. According to Beckley (22), hydroxy-methyl furfural is formed by the action of mineral acids on carbohydrates, and, on condensation, that compound yields "humus;" hydroxy-methyl furfural was also demonstrated in the rotting of straw and in the soil, but not in the decomposition of cellulose by pure cultures of bacteria.



4. The formation of "humus" from lignins. The probable rôle of incrusting substances in the formation of "humus" in the soil has been suggested by various investigators during the last decades of last century; some even considered lignins as the chief sources of "humus" (106). Lange (127) and Schwalbe (205) also expressed the idea that humic acids are closely related to the lignic acids. According to Hoffmeister (105) dark colored substances are formed by the extraction of lignin with alkali, giving finally "humic acids." Dehérain (49) records the results of Hébert who found that when natural organic matter is added to the soil, the sugars and dextrins disappear rapidly and completely; the celluloses and hemicelluloses diminish appreciably whereas the lignins are attacked least. Dehérain adds that the lignin is soluble in alkalies and forms the "matière noire." The nitrogen part of the black material ("humus") is derived from ammonia which is assimilated by microorganisms for the synthesis of proteins, the available carbohydrates being used as a source of energy. "Humus" thus represents a mixture of lignins and proteins. Such a clear definition of the problem of "humus" formation has been altogether overlooked by subsequent investigators on the subject.

Wehmer (240, 241) found that, in the degradation of wood by fungi, especially certain higher fungi, the lignocelluloses are attacked, the celluloses disappearing and the lignins becoming converted into "humic substances." He suggested that, in peat and coal formation, the plant tissues are first converted into "humic" substances by the fungi and not by the bacteria; the lignins, however, cannot be attacked by fungi. According to Trusskov (231), celluloses, hemicelluloses, lower saccharides, and glucosides take no part in the formation of "humus;" lignins, proteins, pigments and tannins give rise to the typical black "humus." Fats and waxes do not change into "humus," but because of their slow decomposition, they form a



part of it. The results of Bray and Andrews (37) and of Du Toit (54) on the decomposition of wood by different fungi tend to demonstrate further that when organic matter is acted upon by microorganisms, the celluloses are rapidly decomposed, the pentosans may remain partly undecomposed, and the lignins remain intact or are acted upon only to a very limited extent.

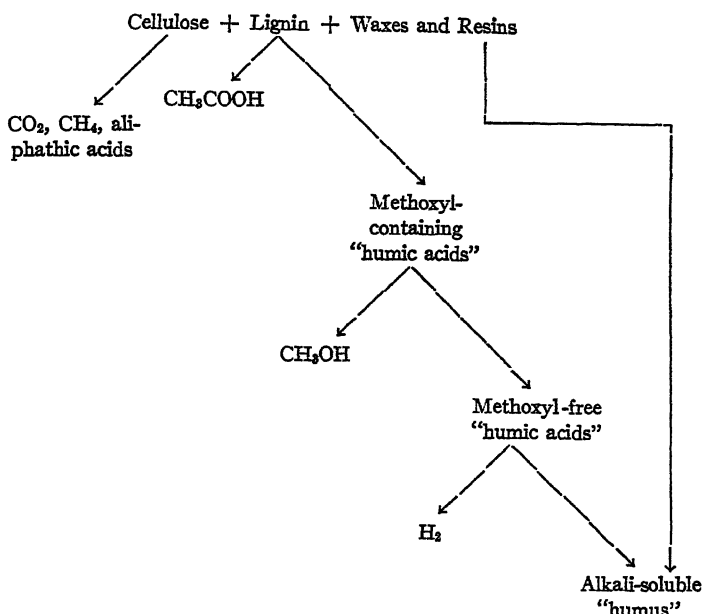
The most extensive work on this subject is that of Fisher and his associates (70, 71), who submitted a very interesting thesis concerning the important rôle that lignins play in the formation of "humus." They have pointed out that cellulose has an aliphatic or furan-like structure, but that lignin has an aromatic structure containing the benzol ring with acetyl and methoxyl groups. When plant substances are decomposed the celluloses change to  $\text{CO}_2$  and water, whereas the lignin remains and accumulates in peat materials; as a result of the breaking off of the acetyl group, the phenol-containing alkali-soluble body of lignin is changed to "humic acids." By an increase in the size of the molecule, either by oxidation or dehydration, the "humic acid" is changed into insoluble "humin," and then into coal. This theory was substantiated by the following considerations:

	 (FURAN- DERIVATIVES)	 (BENZOL- DERIVATIVES)
Cellulose.....	+	—
Lignin.....	—	+
Sugar.....	+	—
Artificial "humus" from sugar.....	+	+
Natural "humus".....	—	+
Coal.....	—	+

By inoculating with a soil suspension cellulose, lignin, wood, and sphagnum moss, placed in an inorganic nutrient solution and incubating at  $37^\circ$ , fungus development took place in the moss, and later in the wood and cellulose but not in the lignin prepared by Willstätter's method. These and other considerations led Fisher (69) to conclude that lignin is the mother substance of natural "humic acids." Fischer suggested that when natural organic substances are decomposed (under conditions of peat formation), the processes on following page take place.

Under favorable conditions, the "humus" loses water, methane, and carbon dioxide, giving bituminous coal together with the resins and waxes.

A decrease in the cellulose content of peat during decomposition and with age of material has been observed by v. Feilitzen and Tollens (68) and by Kerpeler (117), and a considerable increase in the proportion of the organic material soluble in alkalis, referred to as "humic acids," in the older layers of peat has been recorded by Schneider and Schellenberg (200). Thus the formation of peat can be looked upon [Thiessen (228)] as the decomposition of natural organic matter under anaerobic or water-logged conditions, whereby the soluble carbohydrates, hemicelluloses, and celluloses are decomposed, while the lignins and the protective substances (waxes, etc.) accumulate. Under those conditions, the fungi are eliminated while the cellulose-decomposing bacteria are found in abundance. The type of peat formed will depend on the completeness with which the celluloses are disintegrated; most peats (fibrous peats) still contain some undecomposed celluloses. "Humus" compounds obtained from peat can be separated, on the basis of their chlorine derivatives, into two different types, one of which is similar to a lignin compound and one to "artificial humus" obtained by the action of mineral acids on carbohydrates or from cellulose fibres decomposed through aging. It has been suggested that the second compound present in peat is due to decomposition of cellulose by chemical agencies (225). Assuming that the silica content of saw grass



was not diminished during peat formation, Miller (148) calculated that seven parts of the saw grass were required to yield one gram of peat, whereby 33 per cent of the original nitrogen was lost. Other recent contributions (170, 189) tend to substantiate the theory concerning the rôle of lignin in peat and coal formation. Rose and Lisse (189) found that the decomposition of wood goes hand in hand with a decrease in the cellulose and an increase in the lignin content, as determined by the methoxyl ( $\text{CH}_3\text{O}$ ) groups characteristic of lignin [Zeisel method (251)] and of alkali-soluble material:

WOOD	CELLULOSE	METHOXYL GROUPS	ALKALI-SOLUBLE MATERIAL
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Fresh. ....	59.0	3.9	10.6
Half decomposed. ....	41.7	5.2	38.1
Completely decomposed. ....	8.7	8.7	65.3

Similar results were obtained by Balks (17), by Fischer, Schrader, and Friedrich (71) and by König (120a). The presence of methoxyl in the organic matter of mineral soils has been demonstrated by Shorey and Lathrop (210). The results obtained by Dore and Miller (53) on the decomposition of wood by *Teredo* are of interest in this connection; a chemical analysis of the wood borings ejected by *Teredo* and of the original wood from which the borings were derived gave the following results:

	SERIES I			SERIES II	
	Wood (parts per 100)	Borings 1 (parts per 56.1)	Borings 2 (parts per 54.7)	Wood (parts per 100)	Borings (parts per 50.9)
Hemicellulose. ....	6.02	3.62	5.10	14.23	6.20
Cellulose. ....	57.74	11.79	10.99	47.45	10.96
Lignin. ....	30.60	30.60	30.60	27.84	27.84
Furfural yield. ....	5.37	3.32	3.32	5.90	4.26

These results indicate definitely that the *Teredo* utilizes readily the celluloses and hemicelluloses, but not the lignins. König (120a) has shown that in the digestion of plant tissues by herbivorous animals, the celluloses are attacked much more rapidly than the lignins.

None of these four theories fully explains the nature of soil organic matter commonly referred to as "humus" or even the part soluble in alkalis and precipitated by hydrochloric acid, referred to as "humic acids." The first three theories assume the existence of strong mineral acids, soluble carbohydrates, or free amino acids. None of these can exist in the soil for a very long time. Mineral acids would not be produced in more than mere traces in any normal soil, and will then immediately be converted into salts by the soil bases. Soluble carbohydrates will be attacked immediately in the soil by the numerous fungi and bacteria in the presence of available nitrogen or by nitrogen-fixing bacteria, in its absence. Free amino acids will be acted upon immediately by the numerous soil organisms which will utilize them either as sources of nitrogen, energy, or both, or for the structure of their protoplasm. The formation of mere traces of "humus" is possible by these processes. The fourth theory has sufficient basis to deserve careful study and consideration. The similarity in physical and certain chemical properties, of the lignins and the "humic acids" and the fact that neither are readily acted upon by microorganisms give weight to this theory. Both lignins and "humic acids" are soluble in alkalis and precipitated by hydrochloric acid, both contain methoxyl groups, both darken on oxidation, both are acted upon by  $H_2O_2$ , and both absorb water. The theory that lignins are the mother substances of soil "humus" fails, however, to account for one very important constituent of this humus, namely the nitrogen. Lignin is free from nitrogen, whereas the "humic acids" prepared from peat and mineral soils always contain 2 to 4 per cent of nitrogen (usually 3 per cent). Moreover, the existence of a constant ratio between the carbon and nitrogen content of the soil has been definitely established. It is true that this nitrogen is unevenly distributed among the soil fractions separated by alkali treatment, as shown by Berthelot and André (29), but the ratio between the carbon and nitrogen seems to be balanced for the soil as a whole, ranging usually from 12:1 to 8:1. It is not sufficient and hardly scientific to explain this phenomenon by the fact that nitrogen is present as an impurity of the "humic acids" in the soil, as was done by Detmer (50), Eller (64, 65) and Oden (162), simply because the nitrogen content did not fit into the particular formula for the supposedly pure "humic acid." It is true that the soil nitrogen is present in a complex organic form, although probably not as free protein, otherwise it would be rapidly decomposed by the soil microorganisms. These nitrogen complexes (222) either are not readily acted upon by microorganisms or only by certain specific forms that develop only under certain conditions, or they are closely connected with a non-nitrogen group of a high carbon content, the complex as a whole not being attacked by the great majority of soil microorganisms. Once the freshly added organic matter has decomposed rapidly, the remainder decomposes rather slowly. The nitrogen is

liberated as ammonia and then oxidized to nitrate, while the carbon is liberated as  $\text{CO}_2$ , the ratio between the combined carbon and nitrogen in the soil remaining always constant, at about 10-1. Eller and Oden reduced somewhat the nitrogen content of their humic acids only after repeated treatments with strong alkali. This may indicate that the nitrogen-bearing fractions of the soil organic matter can be to some extent more readily hydrolyzed than the bulk of the non-nitrogen bearing fraction. As a matter of fact, Berthelot and André (30) and others also found that the treatment of the soil with alkalis brings into solution a proportionately much greater amount of nitrogen to carbon than is left in the soil or than can be precipitated out by hydrochloric acid. But this is hardly sufficient reason to assume that definite chemical compounds exist in the soil as "humic acids" which are free from nitrogen, and that the nitrogen is present only as an impurity. The lignin theory does not account for this nitrogen and its relation to the other soil organic constituents.

It is also important to note, in this connection, that "humus" actually may accumulate in the soil, but, when a soil is properly limed and well aerated, the "humus" disintegrates and gradually disappears. This indicates that a certain set of conditions, as in the case of peat soils, favors the accumulation of humus, whereas another set of conditions, as found in normal cultivated soils, favors the slow gradual decomposition of the "humus." Gehring (81) found, for example, that when soil is fallowed the total organic matter is somewhat reduced, in comparison with the same soil receiving stable manure or clover. This is accompanied by a very considerable reduction of that part of the organic matter which can be oxidized by hydrogen peroxide. This points to the existence of organisms, which, under definite conditions, are capable of decomposing the "humus." These discrepancies can be explained on the basis of the fifth theory concerning the origin of "humus."

5. *The formation of soil "humus" as a result of the synthesizing activities of microorganisms.* According to Post-Ramann (175) and Müller (155), the "humus" bodies obtained from soil often consist of chitinous remains of insects and animal excreta. Kostytscheff (124) and Ollech (164) suggested that these "humus" bodies may often be the remains of bacteria and fungi. Wettstein (244) and Winterstein (249) demonstrated that chitin is characteristic of various fungi and not of bacteria. Schmook (208) advanced the idea that protein nitrogen in the soil was largely present in the bodies of bacteria and protozoa. Trussov (231) also demonstrated that the protoplasm of fungi and probably also of bacteria serves as a source of "humus" in the soil; according to this investigator, all organic substances can thus become indirectly sources of "humus" in the soil, after passing through the bodies of the microorganisms. "Humic acid" preparations often contain large amounts of waxes and resins (195); these may come partly from the natural organic matter and partly from the bodies of microorganisms. Schreiner and Shorey (204) suggested that various chemical substances found to be characteristic constituents of the soil are probably synthesized by microorganisms. These facts are supplemented by other information submitted by various investigators (120, 23, 241, 95) concerning the rôle of microorganisms in the formation of soil organic matter or soil "humus."

Falck (66) differentiated several processes of transformation of organic matter in forest soils, leading to the formation of different types of "humus": (a) The complete decomposition of organic matter by fungi (*mycoecriny*), whereby the yearly addition of raw materials is

balanced by the amount of decomposition taking place, without any increase in "humus" content. Fungus protoplasm is hereby synthesized; this serves as excellent fertilizer for the forest trees. The celluloses are decomposed completely, whereas the lignins are more resistant; in some cases (as corrosion by Basidiomycetes) however, the lignins and cellulose are both completely decomposed. (b) Decomposition of organic matter is begun by fungi and then interrupted by lower invertebrates and bacteria (*anthracriny*). The fungus mycelium and the original organic matter is devoured by larvae of various insects and worms, producing a dark "humus" mass which is then attacked by bacteria in the presence of basic materials, the action resulting in the liberation of carbon as  $\text{CO}_2$  and nitrogen as ammonia then nitrate. These processes result in the formation of a muck soil. (c) The process of peat formation (*anthrogeny*) is less clearly understood. Falck explains this process by the absence of abundant fungus development.

The author (236) called attention to the similarity between the carbon and nitrogen ratio in the soil organic matter and in the protoplasm of soil microorganisms (largely fungi) and suggested that these probably make up a large part of the soil "humus." When cellulose is added to the soil, it decomposes only in proportion to the available nitrogen contained in the soil liberated by it in a certain period of time (99, 238). This is because cellulose is decomposed in normal soils largely by fungi and also by bacteria, both of which require combined nitrogen. The ratio between the amount of cellulose decomposed and the nitrogen required will be about 30 to 1; i.e., for every 30 parts of cellulose decomposed by the fungi or bacteria, one part of nitrogen will be changed from an inorganic form, like ammonium salt or nitrate, to microbial protoplasm. In the presence of sufficient nitrogen, the decomposition of cellulose by pure cultures of aerobic microorganisms takes place very rapidly. The same is true not only of cellulose but of straw, wood products, corn stover, and other natural substances rich in celluloses, pentosans, and lower carbohydrates and poor in nitrogen. This phenomenon accounts largely for the injurious effect of straw on plant growth. This large amount of nitrogen is needed because above 30 per cent of the carbon of the cellulose which has been decomposed may be changed into carbon of microbial protoplasm. This involves the synthesis of considerable amounts of protoplasm, which results in the storing of large quantities of nitrogen. The constant synthesis of proteins and other complex organic nitrogenous substances by microorganisms in the soil, whether carbohydrates or proteins are used as sources of energy, has been recorded by Dehérain (49), Lathrop (129) and others, as noted above.

While the lignins may contribute the bulk of the soil organic matter, the microorganisms through their synthesizing activities may contribute the nitrogen part of this organic matter

### *Decomposition of "humus" by microorganisms*

If little is known concerning the nature of the soil organic matter, if the origin of these dark colored organic substances in the soil is little understood, the amount of knowledge concerning the decomposition of this "humus" either by microorganisms or by chemical agencies is certainly very limited. Conflicting statements, vague generalizations not based upon any experimental data, a number of speculations, are all that can be found in the voluminous literature on the transformation of organic matter in the soil. On the one hand statements are made (100, 101, 102) that all organic matter must pass through the "humus" stage before its nitrogen can become available to higher plants; also that soil "humus" can even be used as a source of energy for nitrogen-fixing bacteria (135, 133). On the other hand, the few data obtained by careful experimentation (159, 178, 120) indicate that "humus" is a highly resistant substance and cannot be readily acted upon by microorganisms.

Hoppe-Seyler (106) stated as far back as 1889 that, although "humic acids" afford a habitat to various animals, fungi, algae, and bacteria, no plant or animal is capable of obtaining nutrition from them and no bacterium is capable of decomposing them. Schmidt (197) found that pure "humic acid" cannot serve as a source of energy for *Azotobacter* or other bacteria. Sphagnum moss and young sphagnum peat can serve as an indirect source of energy, because of the presence of carbohydrates which can be hydrolyzed with dilute acids.

A careful study on the utilization of "humic acids" by fungi was made by Reinitzer (178). The "humic acid" was obtained by treating the soil with a dilute ammonia solution at 30 to 40°C. The liquid was removed and concentrated on a water bath. The residue was again extracted and the filtrate added to the first extract. The concentrated solution was precipitated with hydrochloric acid. The precipitate was redissolved in ammonia and the excess of the latter was removed by concentrating the solution on a water bath. This deep brown solution or the hydrochloric acid precipitate was used as a source of "humus;" it contained nitrogen, potassium, phosphorus, magnesium, and sulfur, sufficient for the growth of the organisms. *Pen. crustaceum* Fries readily developed upon these humus preparations. Reinitzer recognized quite correctly, however, that the methods employed for the extraction of the "humus" from the soil resulted also in bringing into the solution various carbohydrates, such as pentosans, hemicelluloses, pectins, and gums, even if chitin, cellulose, fats, and waxes were not extracted by the dilute ammonia. These carbohydrates, which were also precipitated by the acid, probably served as a substrate for the growth of the fungus. To avoid this, the "humus" preparation was boiled for one or two hours with 5 per cent HCl to hydrolyze the carbohydrates; the residue was washed, redissolved in ammonia, and the excess of the latter removed on a water bath. No fungus development took place on the "humus" so treated, whether in solution or in a precipitated form. On repeating these experiments with decayed wood, exactly similar results were obtained, the crude preparations (lignin in case of wood, "humus" in case of soil) gave some growth of fungi, but when treated with 5 per cent HCl no fungus development took place. The "humus" could be used, however, as a source of nitrogen by microorganisms.

Nikitinsky (159) treated soil with 10 per cent HCl, then washed the soil with water and extracted it with 10 per cent NaOH; the extract was precipitated with HCl, giving "humic acid;" this was washed with HCl and water and dried. It contained 3.22 per cent nitrogen, 4.92 per cent ash, and 51.56 per cent carbon. Some of the "humic acid" was dissolved in ammonia and the "humate" dried on a water bath. Four grams of "ammonium humate" was added to 400 gm. quartz sand to which also 60 cc. of a solution containing 0.2 per cent  $\text{KH}_2\text{PO}_4$  and 0.2 per cent  $\text{MgSO}_4$  was added. The flasks were sterilized, inoculated with soil suspension, and connected with a respiration apparatus. Only about 4 per cent of the carbon was changed to  $\text{CO}_2$  in 9 days by certain aerobic organisms. Nikitinsky (159) came to the conclusion that

humates cannot be used as sources of carbon by microorganisms but can be used as sources of nitrogen.

Robertson, Irvine, and Dobson (184) prepared natural "humic acids" from peat, by extraction with 5 per cent NaOH, then precipitating with excess of strong acid. The product thus obtained contained 57.14 per cent carbon and 2.79 per cent nitrogen. Microorganisms made only a small amount of growth when this preparation was used as a source of carbon and  $\text{KNO}_3$  as a source of nitrogen, and a much better growth with peptone as a source of nitrogen. Robertson and associates suggested, therefore, that their results were contrary to those of Nikitinsky. The former investigators, however, used crude "humic acid," which was found by Reinitzer to allow some growth of fungi because of the presence of impurities. When peptone was added, the improved growth was no doubt due to the utilization of the peptone and not of the "humic acids" as a source of energy.

There is no doubt, however, that there are organisms in nature which are capable of decomposing even purified "humus" preparations, otherwise "humus" would constantly accumulate in the soil; it is known that well aerated and limed soils readily lose their organic matter. Continuous cropping of land, especially with summer fallowing was found to lead to a decrease in the "humus" content of the soil, as shown by Snyder (215), Alway (6, 7, 8, 9, 10, 11, 12), Ladd (126), and numerous others. Rosenberg (190) considered certain cocci to be capable of decomposing "humus." Salzmann (192) and Störmer (219) found that actinomycetes can obtain nourishment from peat extracts and from raw "humus." According to Nikitinsky (159), various cocci, rods, and actinomycetes are capable of decomposing "humus," but not under anaerobic conditions. Chitin, which was found to be a common constituent in the soil and in the cells of certain soil organisms can also be decomposed by certain bacteria and actinomycetes. Winogradsky (248) recently demonstrated that when "humus" extract is added to a silica gel plate and inoculated with soil, certain bacteria ("autochthonous" organisms) develop out of the soil, using the "humus" as a source of energy and nitrogen.

Agafonoff (3) pointed out that, with a definite set of conditions, a definite equilibrium is established between the accumulation of "humus" in the soil and its decomposition. White and Holben (246) found that when soil is treated with lime and manure for a period of 40 years, 90 per cent of the organic matter is decomposed as compared with 84 per cent decomposition of the organic matter added to the soil in the form of manure but without lime. The manured soil treated with lime contained less readily soluble humus than the unlimed manured soil. It is also commonly reported that fallowing of soil results in a diminution in the amount of available "humus."

According to Ehrenberg and Bahr (60, 61), forest "humus" without lime, has an injurious effect upon the growth of economically important crops. Stüchting and associates (222) found, however, that forest humus does not prevent the assimilation of added available nitrogen compounds by higher

plants. In 2 years, 3.25 per cent of the nitrogen of forest "humus" was made available when treated with lime. In ordinary forest soils, the nitrogen is probably made available to the trees by the interaction of the mycorrhiza fungi (144). It is sufficient to point also to the common practice of draining and liming peat soils to bring about the liberation of the nutrients. A change from anaerobic to aerobic conditions and from an acid to a less acid reaction of the soil brings about the development of organisms capable of decomposing the organic matter of peat.

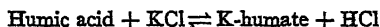
The stimulating influence of "humus" upon the activities of microorganisms has been studied in connection with the growth of *Azotobacter* and nitrogen-fixation. Krzemieniewski (125) first observed that crude "humus" stimulated this process. Fischer (73) recorded the same stimulating influence upon other bacteriological processes and ascribed this to the action of "humus" as an oxygen carrier. Löhnis and Green (135) ascribed the stimulating action to the chemical improvement of the medium. "Humus" was also reported to exert a favorable influence on nitrification (179); this influence was believed to be due to the iron content of the "humus." In view of the fact that no differentiation has been made between the total soil organic matter and the alkali soluble fraction, it is impossible to point definitely to any specific substance, to which the favorable effect upon the growth of microorganisms is due. Further information on the decomposition of forest "humus" by microorganisms is given by Hesselmann (97) and by Möller and Hausendorf (149).

### *The rôle of "humus" in soil processes*

The important rôle of soil organic matter (or "humus") in soil fertility is well recognized. It is sufficient to mention:

1. The physical properties of the soil organic matter in influencing tilth, moisture, temperature, and the nature of the soil solution.

2. Its chemical properties of combining with soil bases, interacting with various salts. It exerts thus an important influence upon the reaction of the soil, either acting directly as a weak organic acid or by combining with bases liberating the more highly dissociating inorganic acids (235). According to the Daikuhara (47) and Kappen (115), the soil acidity is a result either of the interaction between neutral salt solution and soil "humus," due to the liberation of inorganic acid:



or between aluminum and iron humates with neutral salts, by a process of base exchange. The effect of "humus" in the weathering of silicates is also important in this connection (160). The presence of a considerable amount of nitrogen and minerals, necessary for the growth of higher plants in this organic complex makes it economically of the greatest importance.

3. The biological properties of "humus" in offering a habitat and a source of energy, nitrogen and minerals for various microorganisms (15).

The ability of soil "humus" to absorb bases was first studied in detail by Eichhorn (62). The influence of this phenomenon upon the solubility of phosphates has also been pointed out by Fleischer (74), although Sprengel (217) was the first to demonstrate that phosphates are decomposed by natural



"humic acids." Different phosphates are decomposed to a different extent, different soils and peats producing a different action. The addition of certain salts like KCl and especially  $K_2SO_4$ , stimulates this process, whereas others, like gypsum, injure it. When prepared "humus" is brought in contact with insoluble phosphates, a large part of the latter is changed to monocalcium phosphate and sometimes phosphoric acid (19). As much as 0.56 to 0.98 gm. of phosphoric acid was set free from 15.7 gm. calcium phosphate, when the latter is mixed with 100 gm. of peat. The greater the amount of water available for a given amount of peat and phosphate, the greater is the amount of phosphoric acid brought into solution. This solubilization of the phosphate is due to the adsorption of the base and the liberation of the acid. Sphagnum itself acts as an acid, absorbing calcium from  $CaCO_3$  (123).

It has been shown repeatedly (1), that the acid reaction of sandy and peat soils is caused by "humic acids." The assumption is thereby made that this "humic acid" of both soil types is the same, has the same equivalent weight and probably the same chemical composition. According to Oden (162, 163) the favorable effect of liming acid and bog soils is due to the neutralization of the toxic acids and the formation of "humates" which act as buffers, opposing the formation of injurious acids. The neutralized organic substances are also more readily decomposed by microorganisms, making the nutrients available for plant growth. The soil "humus" acts as a buffer not only to changes in reaction, but in its effects upon tilth, moisture, soil temperature, and soil solution, preventing harmful extremes and making mineral soils stable for cropping purposes (168). Weir (242) claimed that by removing 40 per cent of the soil nitrogen with sodium hydroxide, and using the residual soil for vegetative experiments, approximately the same yields were obtained as from the untreated soil. However, Crowther (44) reported later that the removal of the "humus" by alkaline extraction from both a garden and a field soil reduced the productiveness over a series of crops in pot experiments. The initial increase observed in field soil was only temporary.

The amount of "humus" in the soil varies not only with depth but also with age of soil (200). Soil treatment influences the amount of "humus" in the soil, but does not change materially the ratio of the carbon to the nitrogen (96, 41). This carbon-nitrogen ratio is higher, however, in surface soil layers than in the subsoil (55, 10). Liming was found to hasten the decomposition of the soil organic matter (93, 173, 245) in peat and in mineral soils.

Without going further into a discussion of the literature on the rôle of organic matter in the soil, it is sufficient to state that it lies at the very base of our agricultural practice and soil treatment.

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## THE ABSORPTION OF IRON BY SOILS

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Iron is one of the elements which is present in soil in large quantities and is required by growing plants in almost infinitesimally small quantities.

It has long been known that solutions of ferrous salts are definitely toxic to plants, and that such solutions persist for only a very short time in normal soils, being oxidized with great rapidity and producing a harmless ferric salt which is retained. This retention is due to the fact that when a solution of a ferric salt is in contact with soil the iron is usually completely precipitated. This fact is made use of by Comber (1) in his method for distinguishing acid from neutral soils.

The work described in this paper was originally suggested by observations contained in a previous paper (2), which it was desired to investigate more fully. It has been shown by one of the authors (3) that it is not possible to distinguish with certainty the presence of ferrous compounds in soil, and that only when the concentration of the soil solution becomes abnormally low does there appear to be any movement of iron either in the form of a true solution or as a colloid sol. The object of the present work is to show: (a) the extent to which iron is removed from solution by various soils and (b) the soil constituents which are concerned in the removal.

In order to investigate the absorption of iron by various soils, 10 gm. of air-dried soil was placed in contact with 100 cc. of ferric chloride solution, containing 0.1482 gm. of iron. The mixture was kept in a flask and frequently shaken by hand for 15 hours. The contents of the flask were then filtered and the iron was determined in 25 cc. of the filtrate by a volumetric method which briefly consisted in:

(a) conversion into ions of any colloidal iron by boiling with a few cubic centimeters of concentrated sulphuric acid; (b) complete reduction of the iron from ferric to ferrous state by addition of a solution of stannous chloride; (c) removal of excess of stannous chloride by addition of concentrated mercuric chloride, which was reduced and precipitated as mercurous chloride; (d) determination of the amount of ferrous iron by titration with decinormal potassium bichromate using potassium ferricyanide as an external indicator.

The results of this experiment are given in table 1.

Of these soils there are three which show complete removal of iron from the solution used, and all of these contain considerable quantities of calcium carbonate, which would thus appear to be in part responsible.

TABLE 1  
*Absorption of iron from solution of ferric chloride by various soils*

NUMBER	FORMATION	DISTRICT	TYPE OF SOIL	IRON ABSORBED BY 10 GM. SOIL  <i>per cent</i>
C.	Kimeridge clay	Sandford, Oxon	Clay*	100.0
D.	Valley gravel	Oxford	Loam*	100.0
E.	Kimeridge clay	Sandford, Oxon	Clay*	100.0
P.	Alluvium	Otmoor, Oxon	Very heavy clay	71.5
Q.	Alluvium	Otmoor, Oxon	Very heavy clay, sub- soil of P.	65.4
V.	Marlstone, Middle Lias	Adderbury, Oxon	Sandy loam,* high iron content	64.5
S.	Marlstone	Windrush Valley, Oxon	Sandy loam,* high iron content	58.8
R.	Marlstone	N. Oxon	Sandy loam, high iron content	56.9
L.	Calcareous grit	Fyfield, Berks.	Sand*	54.2
U.	Chalk	Assenden, Oxon	Sandy loam*	53.5
T.	Forest marble	Leafield, Oxon	Clay	50.4
B.	Kimeridge clay	Sandford, Oxon	Light clay	30.7
H.	Kimeridge clay	Bagley Wood, Berks.	Sandy clay subsoil	19.5
A.	Old red sandstone	Llanthoney, Mon- mouthshire	Sandy loam	9.2
G.	Plateau gravel	Tadley Common, Hants.	Sand	9.1
M.	L. greensand	Boar's Hill, Berks.	Sand	3.8
N.	Calcareous grit	Tubney, Berks.	Sand	4.6
O.	Calcareous grit	Tubney, Berks, sub- soil	Sand, subsoil of N.	1.0

\* No lime requirement.

Soils P and Q are from Otmoor, one of the heaviest soils in England, containing more than 40 per cent of clay but showing a positive lime requirement, and yet the absorption in this case is 71.5 for the surface and 65.4 for the subsoil.

The next group consists of three soils from the Middle Lias formation, containing very high amounts of iron. Only one of these soils shows a lime requirement, though all have a high iron absorption. The absorption in this case must be mainly due to something other than calcium carbonate.

Soils L and U show no lime requirement and contain appreciable quantities of calcium carbonate (0.25 per cent and 0.4 per cent respectively), and the remainder, all showing a positive lime requirement, have an iron absorption varying from 30 per cent in the case of clay soils to 1 per cent in the case of sands.

These results are sufficient to show that although calcium carbonate is an appreciable and important factor in the absorption of iron, other factors also contribute. There appear to be four such factors in soil: (a) calcium carbonate, (b) gross amount and activity of clay, (c) ferric oxide, (d) organic matter.

TABLE 2  
*Absorption of iron by soils at different depths*

	CaCO <sub>3</sub>	Fe REMOVED FROM 100 cc. 0.1 N FeCl <sub>3</sub> BY 10 GM. SOIL
	<i>per cent</i>	
First inch*.....	0.2508	56.3
Second inch.....	0.1672	54.2
Third inch.....	0.2090	54.2
Fourth inch.....	0.2132	54.2
Fifth inch.....	0.1923	53.5
Sixth inch.....	0.1044	50.0
Seventh inch.....	0.1754	50.0
Eighth inch.....	0.0334	43.6
Ninth inch.....	0.0417	40.2

\* Top.

#### EFFECT OF CALCIUM CARBONATE ON IRON ABSORPTION

Additions of calcium carbonate to a solution of ferric chloride produce a change in colour of the solution from yellow to orange-brown, with evolution of carbon dioxide until there is sufficient calcium carbonate present to precipitate all the iron as ferric hydroxide, when the solution becomes completely free from iron. The change in colour is due to the formation of colloidal ferric hydroxide, which remains dispersed until the whole of the hydrochloric acid is removed, when it is precipitated as ordinary ferric hydroxide. The effect of calcium carbonate on iron absorption can be shown in the following experiments:

(a) In order to reduce the amount of calcium carbonate present 10 gm. of soil E was treated with 250 cc. of 0.5 N acetic acid and left to stand for 12 hours. The soil was then filtered and washed, transferred to a flask, and treated with 100 cc. 0.1 N ferric chloride solution. After the treated soil had been shaken at intervals for 16 hours, the amount of

iron absorbed was found to be 47 per cent whereas the original untreated soil absorbed 100 per cent. Presumably the removal of calcium carbonate by the acid treatment had some effect in lowering the iron-absorbing capacity of the soil.

(b) Soil U (poor arable field on chalk) was obtained in inch depths down to 9 inches. The calcium carbonate in each inch was determined by means of a Collins calcimeter, and the iron absorption of each inch was also determined.

Results are shown in table 2.

The percentage of iron removed is more uniform than would have been expected with the varying amounts of calcium carbonate, but there is no necessary uniformity in the other soil constituents, and doubtless the clay and humus play some part.

It is clear, however, that in the eighth and ninth inches where the calcium carbonate content is much decreased, iron absorption is also diminished.

Experiments by Comber (1) show that by treating a soil deficient in calcium carbonate with a solution of potassium thiocyanate a red colouration of ferric thiocyanate is observed. Presumably in the presence of calcium carbonate any iron replaced by potassium is immediately reprecipitated by the calcium carbonate.

#### EFFECT OF GROSS AMOUNT AND ACTIVITY OF CLAY ON IRON ABSORPTION

Soils P, Q, T, B, and H absorb respectively 71.5 per cent, 65.4 per cent, 30.7 per cent and 19.5 per cent of iron from solution. As all these show a positive lime requirement, the effect of calcium carbonate on the absorption of iron must be negligible. Presumably the colloidal clay content is the predominant factor, and the proportion of the clay fraction in these soils varies directly with their iron absorbing power, e.g., soil H is far the lightest and soils P and Q the heaviest, the latter containing over 40 per cent of clay.

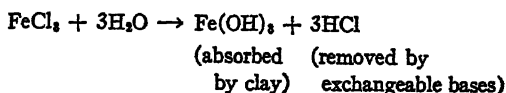
Soil Q, which absorbs 65.4 per cent of iron from solution, was heated in a muffle furnace for 3 hours, after which, 10 gm. of the ignited soil was treated with 100 cc. of ferric chloride solution. Only 5.5 per cent of iron was removed from solution. The ignition of the clay soil destroyed its colloid constituents and consequently reduced its iron-absorbing powers.

The question remains as to whether the process is analogous to the absorption of exchangeable bases, or whether it is due to a mutual flocculation between the clay colloidal material and the colloidal ferric hydroxide that has been formed from the hydrolysis of ferric chloride. The following experiments may throw some light on this point:

Ten grams of soil Q, which shows a lime requirement of 0.1278 calcium carbonate, was shaken with 100 cc. of 0.1*N* hydrochloric acid. The mixture was filtered and 25 cc. of the filtrate titrated with 0.1*N* sodium hydroxide. The result showed that 42.2 per cent of the acid had been neutralized, presumably by the exchangeable bases in the soil.

It is reasonable to suppose that these exchangeable bases could remove some of the hydrochloric acid produced by hydrolysis of the ferric chloride, thereby

causing more ferric hydroxide to be formed, and that this ferric hydroxide would effect a mutual flocculation with the clay colloidal material, i.e., the balance of the equation would be shifted from left to right.



Comber<sup>1</sup> has shown that if a soil is treated with 2*N* hydrochloric acid its exchangeable bases will be removed and its powers of absorbing calcium from a solution of calcium bicarbonate will be considerably increased.

Soil Q was left in contact with 2*N* hydrochloric acid for 24 hours, subsequently was washed free of acid, and was air-dried. The soil was then shaken with ferric chloride solution as before, and it was found that 35.1 per cent of iron was absorbed, whereas the untreated soil absorbed 65.4 per cent of iron.

It may, therefore, be concluded that the absorption of iron by the clay fraction of a soil can be attributed chiefly to the effect of exchangeable bases in producing colloidal ferric hydroxide from the solution of ferric chloride by neutralizing the acid formed by hydrolysis, and that this ferric hydroxide effects a mutual flocculation with the clay.

#### EFFECT OF FERRIC OXIDE ON IRON ABSORPTION

The soils R, S, and V absorb respectively 56.9 per cent, 58.8 per cent, and 65.4 per cent of iron. These soils are very typical sandy loams, and, physically, they are not unlike soil A which absorbs 9.2 per cent of iron; consequently the clay fraction cannot contribute much to the absorption of iron. Their calcium carbonate content is below 0.1 per cent although R alone actually shows a lime requirement, so that calcium carbonate can be ruled out as an active factor in absorption. The predominating feature of these soils is their high iron content, which in terms of ferric oxide is: R, 26.5 per cent; S, 15.7 per cent; and V, 22.5 per cent. The iron in these soils may be in the form of a highly absorptive ferric hydroxide gel, which would appear to be the main factor in the absorption of iron. It is probable that the ferric hydroxide gel in these soils absorbs iron in the same manner as does the clay fraction. It was found that 10 gm. of soil R, which shows a lime requirement of 0.08134 per cent of calcium carbonate, neutralizes 51.2 per cent of 0.1*N* HCl, and that when "emptied" of its exchangeable bases by treatment with 2*N* hydrochloric acid, its iron absorbing powers are reduced from 56.9 per cent to 28.5 per cent.

<sup>1</sup> COMBER, N. M. Note on the absorption of calcium by soils. Paper read before the Conference of Advisory Agricultural Chemists (England). Department of Agriculture, the University, Leeds, England, October, 1924.



## EFFECT OF ORGANIC MATTER ON IRON ABSORPTION

A further factor which must have some influence in iron absorption by a soil is its colloidal organic matter content. At the present stage this has not been investigated to any great extent.

An acid peat was found to absorb 32.6 per cent of iron; N, a sandy forest surface soil, absorbed 4.6 per cent, whereas its subsoil O absorbed 1 per cent. The difference between the heavy clay surface soil P and its subsoil Q is not relatively great, the amounts of iron absorbed being respectively 71.5 per cent and 65.4 per cent.

Probably partly because of the amount of exchangeable bases held, organic matter apparently does play a part in the absorption of iron. This point would be affected by the nature of the organic matter, the extent to which it has undergone decomposition, the rainfall of the district, and the permeability of the underlying soil.

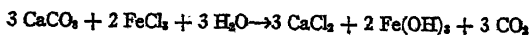
It would appear, however, that each of the four constituents mentioned affect the removal of iron from solution. It should be borne in mind that the Comber method for the detection of acidity depends not only upon the absence of calcium carbonate, but also upon the absence, or comparative scarcity, of the three other constituents. In fact, soils Q and R show a lime requirement of 0.1278 per cent and 0.08134 per cent respectively, but give no red colouration with an alcoholic solution of potassium thiocyanate.

## EFFECT OF INCREASING ADDITIONS OF CALCIUM CARBONATE ON THE IRON ABSORBING POWERS OF SOILS

As stated above, when small additions of calcium carbonate are added to a solution of ferric chloride, a sol of ferric hydroxide is produced which will ultimately be precipitated when sufficient calcium carbonate is present to neutralize all the hydrochloric acid produced on hydrolysis.

In the experiment, 9 flasks were each filled with 10 gm. of soil. To all but the first of these, calcium carbonate was added in regularly increasing amounts of from 0.05 to 4 gm. One hundred cubic centimeters of ferric chloride, containing 0.1482 gm. of iron, was added to the contents of each flask, which was corked up and shaken by hand at intervals for 16 hours. The corks were removed periodically to relieve the pressure caused by the evolution of carbon dioxide. The contents of the flask were then filtered and the amounts of iron in 25 cc. of the filtrate determined.

The results are given in table 3, and are shown graphically in figures 1 and 2. In the tabulation the second column shows the weight of iron present as colloidal ferric hydroxide which would be produced by that weight of calcium carbonate (in the first column) in accordance with the equation



Actually no ferric hydroxide is precipitated by calcium carbonate alone until 0.4 gm. is added, which is sufficient to remove all the hydrochloric acid which can be produced by the hydrolysis of the ferric chloride.

TABLE 3  
*Iron absorbed by soils treated with calcium carbonate*

$\text{CaCO}_3$ ADDED	$\text{Fe as Fe(OH)}_2$ FORMED FROM $\text{CaCO}_3$	Fe ABSORBED BY SOIL P	Fe ABSORBED BY SOIL Q	Fe ABSORBED BY SOIL QX	Fe ABSORBED BY SOIL S	Fe ABSORBED BY SOIL R	Fe ABSORBED BY SOIL B	Fe ABSORBED BY SOIL H	Fe ABSORBED BY SOIL L	Fe ABSORBED BY SOIL U	Fe ABSORBED BY SOIL A	Fe ABSORBED BY SOIL G	Fe ABSORBED BY SOIL M	Fe ABSORBED BY SOIL N	Fe ABSORBED BY SOIL O
gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
0	0	0.1060	0.097	0.052	0.085	0.080	0.0460	0.036	0.080	0.075	0.014	0.014	0.006	0.003	0.002
0.05	0.0186	0.1170	0.115	0.071	0.114	0.101	0.0626	0.047	0.091	0.097	0.028	0.036	0.031	0.022	0.025
0.10	0.0373	0.1360	0.131	0.080	0.133	0.117	0.0840	0.071	0.080	0.103	0.039	0.030	0.019	0.019	0.020
0.15	0.0859	0.1403	0.138	0.099	0.145	0.134	0.1090	0.087	0.080	0.107	0.047	0.032	0.016	0.022	0.018
0.20	0.0746	0.1450	0.146	0.117	0.148	0.140	0.1160	0.105	0.089	0.116	0.052	0.030	0.018	0.020	0.017
0.25	0.0932	0.1460	0.147	0.130	*	0.146	0.1230	0.121	*	0.116	0.052	0.030	0.018	0.024	0.017
0.30	0.112	0.1470	0.148	0.145	*	0.148	0.1370	0.134	*	0.116	0.052	0.028	0.018	0.024	0.017
0.35	0.1305	0.1480	0.148	0.148	*	*	0.1480	0.147	*	*	0.052	0.043	0.018	0.057	0.017
0.40	0.1492	*	*	*	*	*	*	*	*	*	*	*	*	*	*

\* All iron removed.

Graphically the weights of iron absorbed by 10 gm. of soil are plotted against the weights of calcium carbonate added. On each graph, a "normal" is plotted

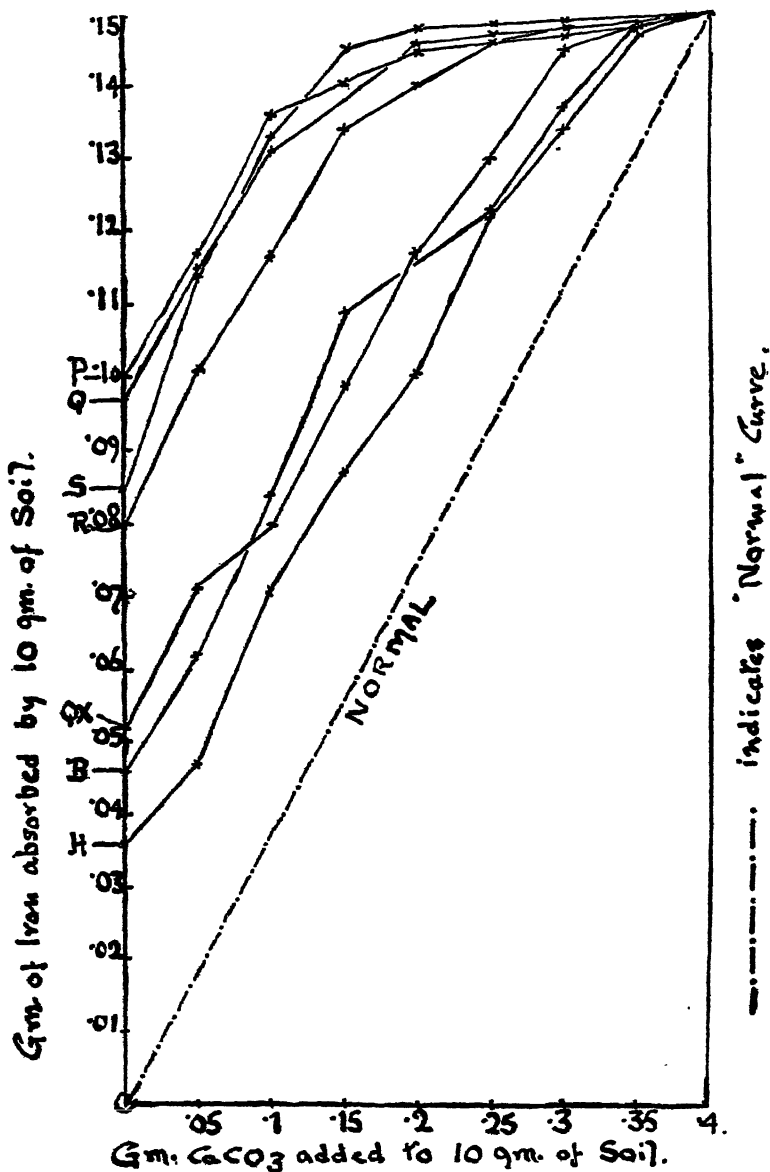


FIG. 1. BEHAVIOUR OF CLAYS AND SOILS WITH HIGH IRON CONTENT

resulting from the first and second columns in the table, i.e., the normal represents the iron as colloidal ferric hydroxide plotted against the weight of

calcium carbonate which would produce it. This curve will be referred to in future as the "normal."

Fig. 1 represents the behaviour of clay soils and soils with high iron content.

In every case, no colloidal ferric hydroxide appeared in the filtrate and there was an increase in iron absorption as the weight of calcium carbonate was increased, so that as soon as any additional ferric hydroxide was formed, it was immediately removed by the colloidal material in the soil. Peculiar flocculation changes were also observed. On the addition of ferric chloride without calcium carbonate, the soil in the flask tended to flocculate. On increasing the quantities of added calcium carbonate, the soil suspensions became more and more turbid up to the point where all the iron was absorbed. At this point a very complete and extraordinarily rapid flocculation took place.

It will be further observed that in the soils P, Q, R, S, the curves are sensibly parallel to the "normal" curve, indicating that the colloidal ferric hydroxide, formed as a result of increasing additions of calcium carbonate, is absorbed by the soil.

The observed differences between the iron concentrations of the solutions in contact with the soil and containing no calcium carbonate, and that containing neither soil nor calcium carbonate, denote the original absorptive powers of the respective soils.

As the concentrations of iron in the solution diminish, the power of the soil to remove iron apparently decreases, and consequently over the latter portions of the curves the slope is more gradual.

The curves for the lighter clays B and H run practically parallel to the "normal" for their entire length, indicating that every successive amount of calcium carbonate is removed by the soil. This means that although the initial absorptive powers of the soils are less than in those discussed above, these powers are maintained over the whole range of concentrations in the experiments.

Soil Q, which gave a curve of the first class, was treated with cold 2*N* hydrochloric acid to remove the exchangeable bases; it was subsequently washed and air-dried, and its absorption determined.

The curve is shown under the heading QX and is seen to be one of the latter class, running roughly parallel to the normal for a considerable distance before it bends toward it, approaching the point where all the iron is absorbed.

The initial amount of iron absorbed where no calcium carbonate is added is also less than that of the untreated soil.

There is not sufficient evidence to determine whether these facts are entirely due to the removal of exchangeable bases. It is quite possible that the treatment with hydrochloric acid would effect some alteration in the soil colloids and that this is the responsible factor.

Figure 2 represents the behaviour of sandy loams and sands. The curves again fall under two heads. Those for the more loamy sands, A and U, gradually approach the normal, intersect it, and become horizontal before all the iron

is absorbed by excess of calcium carbonate. In other words, on the addition of calcium carbonate, absorption of iron by the soil was increased until all or nearly all the clay colloid in the soil was used up; after which, little or no ab-

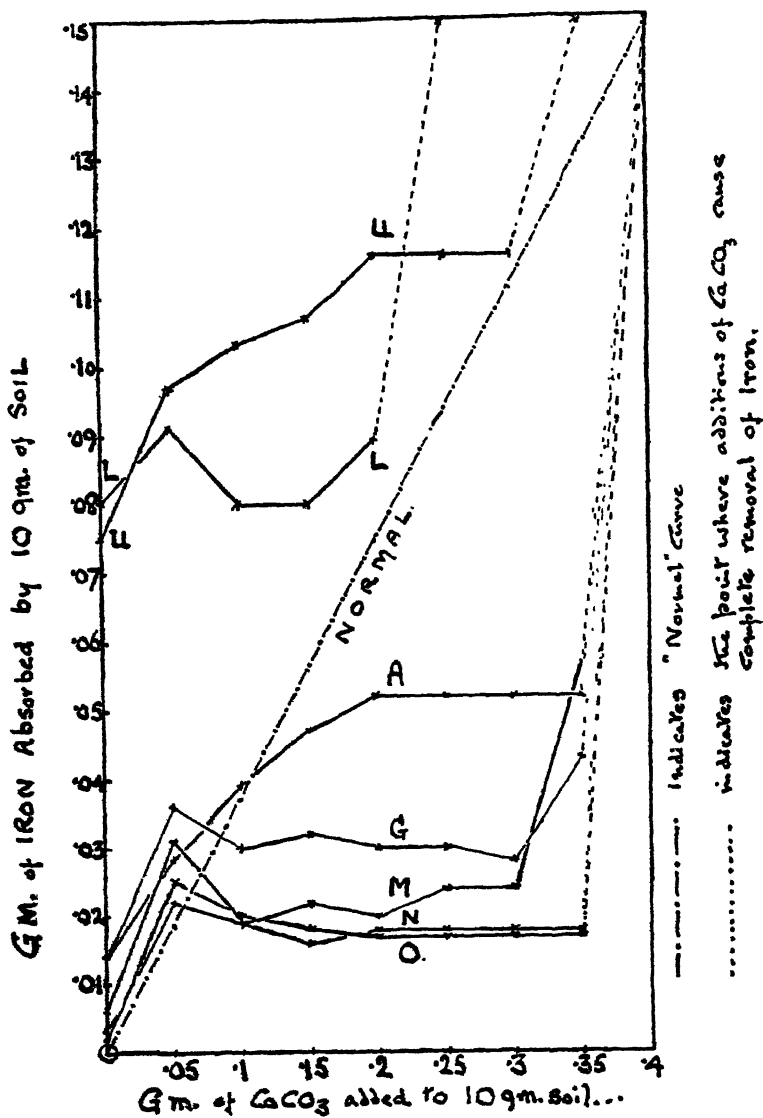


FIG. 2. BEHAVIOUR OF SANDY SOILS AND SANDS

sorption took place until sufficient calcium carbonate was added to precipitate all the iron, whether soil was present or not.

The flattening of the curve was always accompanied by the appearance of

the dark coloured iron sol in the filtrate, showing that the clay colloid in the soil had already been fully "saturated" with iron colloidal material.

It would appear that the maximum absorption of iron by a sandy loam which is deficient in calcium carbonate depends on its clay colloid content, and cannot be increased beyond this point by further additions of calcium carbonate until sufficient has been added completely to precipitate the iron.

Soils A and U behave in this way. It is interesting to observe that the calcium carbonate content of the soil itself has no effect on the type of curve produced when calcium carbonate is added; that is, soil U contains more calcium carbonate than soil A, which shows a lime requirement, and consequently the point where the curve for soil U starts is higher than the similar point for soil A, but the type of curve when further amounts of calcium carbonate are added is the same for both soils, and depends on the colloid content of the soil.

The second type of curve is typical for the behaviour of very coarse sands with the minimum amount of absorbent colloidal material. Such types of curves are represented by soils G, L, M, N, and O. Here again the calcium carbonate content of the soil affects only the starting point and not the type of the curve. (See curve for soil L.)

The curves themselves are peculiar: although the first addition of calcium carbonate raises the iron absorption, the second lowers it, and future additions produce a more or less horizontal curve, showing that no more iron is absorbed until sufficient calcium carbonate is added to precipitate the iron. The addition of 0.35 gm. of calcium carbonate usually had the effect of raising the iron absorption to some extent.

The first part of the curve is more or less parallel to "normal," showing that the absorption is proportional to the ferric hydroxide produced. A "kink" in the curve appears at this point. The "kink point" was always accompanied by intense turbidity and extremely slow filtration, although the soils were the coarsest of sands.

A possible explanation of this "kink point" is that a very thin coating of colloidal material, possibly silica, is held round the large sand particles. The first addition of calcium carbonate produced just sufficient ferric hydroxide to effect a mutual flocculation with all this colloidal material and draw it off the soil particles. Further additions of calcium carbonate produce more ferric hydroxide which would be sufficient to change the sign of the charge on this colloidal material and bring about a mutual *protection* of both colloids in place of a mutual *flocculation*.

It should be noted that similar curves for soil and subsoil are obtained in the case of soils N and O, indicating that the greater organic content of soil N, the surface soil, had little effect on the type of curve produced.

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# THE INFLUENCE OF CALCIUM AND NITROGEN ON THE PROTEIN CONTENT OF THE SOYBEAN PLANT<sup>1</sup>

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## INTRODUCTION

Although the exact rôle that calcium plays in plant nutrition is not yet fully understood, it is becoming more and more evident that this element exerts a controlling influence on soil fertility. That its functions are manifold (30) has been well established by the numerous researches in modern agriculture. Russell (44) states that soils sufficiently supplied with  $\text{CaCO}_3$  stand out in sharp contrast to those containing too little, although they may be otherwise of similar composition. The contributions of Gedroiz (9, 10) have brought out the dominant place that Ca takes in the colloidal complex in the soil. The remarkable antagonism of the Ca ion to the toxicity of other ions in plant growth, has been well demonstrated by Loeb (29), Osterhout (41), McCool (33), and Truog and Sykora (51). Truog (50) suggests that each species of plants has a certain lime-requirement which must be satisfied for maximum growth.

Indeed, the effect of  $\text{CaCO}_3$  on the biological, chemical, and physical changes in the soil are known to the thoughtful farmer. Little is known, however, about the influence of calcium upon the biochemical activities within the plant itself, or concerning its intimate relation to other mineral nutrients in the metabolic processes of the plant. Recent experiments, carried out by several investigators, tend to indicate that a definite relationship exists between the amount of  $\text{CaCO}_3$  in the soil and the nitrogen content of the plants grown in it. Especially is this true for legumes. But just what is the mechanism by which calcium increases the nitrogen content in plants is at present a matter of conjecture. Whether this phenomenon is brought about by direct action of the Ca ion within the plant, or indirectly by the effect of lime on the H-ion concentration of the soil solution, or by its beneficial influence on nitrogen fixation, our present knowledge concerning this subject is too scanty to warrant any definite conclusions.

Furthermore, the results obtained by different investigators in this field are not always in close agreement. Smith and Robinson (48) found that nodules on the roots of cowpeas and soybeans increased both the nitrogen and protein content of plants and seeds but did not increase the total yield. On the other hand Fred and Graul (8) report results which show that inoculation of soybeans increased the yield as well as the nitrogen content and that the yield was still further increased when supplemented by an application of lime. White (54) observed that the calcium content of clover and sorrel was highest where the maximum amount of limestone was applied and that both calcium and nitrogen were higher in the two crops when grown on a neutral or slightly alkaline soil than on an acid soil.

Results obtained by Lipman and Blair from a series of greenhouse and field experiments (22, 23, 24, 25, 26) show appreciable increases in the nitrogen content of soybeans, crimson clover, barley, vetch, and Canada field peas as a result of  $\text{CaCO}_3$  applications to the soil.

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The nitrogen in the legumes was higher with a liberal application of  $\text{CaCO}_3$  than with a liberal application of  $\text{NaNO}_3$ . For a period of three years the percentage of nitrogen was higher in alfalfa hay from limed than from unlimed plots. Stalks, roots, and shelled beans of fourteen different varieties of soybeans grown on limed and unlimed soils were analyzed for total nitrogen. In each case, plants from limed plots were higher in this element and produced larger yields than those from the unlimed plots. Finally, the same authors conclude (27) from results of a ten-year period of crop rotation that the total yield in nitrogen was essentially the same for limed and unlimed sections where crop rotation without legumes was practiced; whereas in rotation with legumes, the plots where  $\text{CaCO}_3$  or  $\text{MgCO}_3$  was applied yielded distinctly larger crops and more total nitrogen than the unlimed plots.

Marked differences in the nitrogen content of red clover were also observed by Morse (35) in favor of the limed plots and were ascribed to higher nitrogen fixation brought about by the added  $\text{CaCO}_3$ . Alfalfa grown on soils ranging in pH from 3.0 to 7.1 was reported by Joffe (16) to increase gradually in nitrogen content with the corresponding decreases in H-ion concentrations. MacTaggart (32) concludes that, as a single element, nitrogen did not increase the nitrogen percentage of soybeans when applied to the soil in the form of dried blood. Experiments in culture solutions, carried out recently (12), have shown that in the absence of Mg, K, P, or Fe, soybean plants absorb low amounts of nitrogen and high amounts of calcium.

Parker and Truog (42), by comparing the composition of some thirty-four different species of plants taken from many different sources, found a direct relation between the nitrogen and calcium content. Higher calcium percentages were (with some exceptions) accompanied by a higher calcium-nitrogen ratio. They further advance the theory that the amount of calcium absorbed is proportional to the protein built up by the plants. Gile and Ageton (11) observed that  $\text{CaCO}_3$  present in the soil in variations of from 5 per cent to 35 per cent had no effect on the amount of nitrogen fixed in the plants when bush beans, soybeans, radishes, sunflower, sugar cane, rice, and sweet cassava were grown. On the other hand, the soil high in  $\text{CaCO}_3$  increased the calcium content only in soybeans, sugar cane, and sunflower. They also found that plants which were most depressed (rice and pineapple) showed the greatest increase in the amount of calcium. This last observation is in close harmony with that previously mentioned (12) although different plants were used.

Newton (39) grew peas, barley, and vetch in solutions low in calcium and found a corresponding decrease of this element in the plants, but no difference in the nitrogen content was observed. Furthermore, inoculated peas absorbed more nitrogen than the uninoculated when grown in solutions low in nitrogen, but did not absorb more calcium. That plants can take in large amounts of calcium without any injurious effects was also shown by Shedd (46). An increase of this element in legume plants occurred with the application of different calcium salts to the soil. This phenomenon, however, does not hold true for all plants and under all conditions. Bryan (4) found that a decrease in acidity from pH 5.0 produced a decrease in the calcium content of oats, but not of wheat plants. He also observed (3) that the greater the acidity of the culture medium, the less is the power of legume plants to obtain calcium for their metabolism.

Thus, all the experiments enumerated here, with only one exception, signify that the application of  $\text{CaCO}_3$  to the soil increases the nitrogen content of plants. Discussion arises, however, whether this increased nitrogen is in protein form or merely as non-protein nitrogen. That this question is of practical importance from the standpoint of food value is obvious. Whereas an increase in protein means more food for human or animal consumption, a higher nitrogen content may not add anything to the food value of the crop. The term "protein" has been rather loosely used throughout the literature on this subject. Many authors speak freely of protein content in plants when only total nitrogen has been determined, overlooking the fact that the nitrogen percentage of the plant, especially of legumes, represents both protein and non-protein nitrogen.

The purpose of these experiments is, therefore, two-fold: First, to determine whether there exists a definite relation between calcium and nitrogen in plant metabolism. Secondly, to

ascertain whether the increased nitrogen found in plants as a result of lime application is in the form of protein or non-protein nitrogen. Solution cultures, in which the ionic concentration of the two elements under consideration can be approximately controlled, seemed to be best adapted to these studies. It was further thought that a comparison of solution-culture plants with those grown on soil submitted to a similar treatment might reveal some interesting correlations. The experimental data recorded in this paper represent, therefore, results secured from both solution culture and soil culture plants.

#### METHODS OF PROCEDURE

The plan of the experiments involved a study of the growth and composition of soybean plants (Manchu variety) grown in culture solutions containing varying proportions of calcium and nitrogen as well as in soil and in soil extracts with and without  $\text{CaCO}_3$ . Soybeans were selected because they are comparatively rich in both elements here considered. By employing soil extracts it was hoped to attain a culture medium in which the conditions for plant growth are more like those prevailing in the soil than could be possibly achieved in synthetic solutions. The entire investigation comprises four groups of plants according to the following treatments:

- Group I. Culture solutions containing varying proportions of calcium and nitrogen in the form of  $\text{Ca}(\text{NO}_3)_2$ .
- Group II. Culture solutions containing equal amounts of nitrogen and varying amounts of Ca as  $\text{CaCl}_2$ .
- Group III. Soil extracts and culture solutions saturated with  $\text{CaCO}_3$ .
- Group IV. Limed and unlimed soils.

The solution plants were grown in the greenhouse in two separate series, designated in the tables as *A* and *B* respectively. The second series was an exact duplicate of the first, but conducted at a different season of the year. In the first series, soybeans were grown from March 17 to August 25, whereas the second series was conducted from July 3 to September 5, 1924. The soil plants were grown during the summer months of the same year. When the plants had matured they were harvested and the dried material was analyzed for calcium, magnesium, protein nitrogen, and total nitrogen. Magnesium was determined with the object of studying the still debated question of the relation between the calcium and magnesium in plants (13, 19, 21, 20, 31, 37). The determination of calcium, nitrogen and protein was required to carry out the purpose of the experiment.

For the culture solution work, a three-salt solution having an osmotic concentration of one atmosphere and containing the three salts  $\text{KH}_2\text{PO}_4$ ,  $\text{Ca}(\text{NO}_3)_2$ , and  $\text{MgSO}_4$  in volume—molecular concentrations of 0.0027, 0.0027, and 0.0161, respectively—was used as a check on the basis of which the other solutions were prepared. Two-quart glass jars were used for culture vessels and were arranged in the manner described by Shive (47) and Jones and Shive (18). Three plants were grown in each jar and duplicate jars were used, thus giving six plants for every solution in each of the two series. The solutions and the soil extracts were continuously renewed by means of a constant drip and drain

method (2) which allowed one liter of new solution to flow into each culture jar during a period of twenty-four hours while an equal amount of solution was automatically removed from each culture. Constant aeration was accomplished in the solutions by means of an air pump especially improvised for this purpose and described by Neller (38) and Allison (1).

Seeds, ranging in weight from 195 mgm. to 205 mgm. were germinated in sphagnum moss and seedlings of uniform size were transferred to the cultures. The culture solutions were prepared as used, from single 0.5 *M* stock solutions, and iron was added, whenever the plants appeared to require it, in the form of a freshly prepared solution of soluble ferric phosphate.

It was conceived from the very beginning that, if an adequate study of this problem is to be made, the composition of healthy plants only should be com-

TABLE 1  
*Osmotic concentrations, pH values, and salt concentrations (in grams per liter) of the culture solutions*

GROUP	CULTURE NUMBER	pH VALUES	OSMOTIC CONCENTRATIONS	KH <sub>2</sub> PO <sub>4</sub>	Ca(NO <sub>3</sub> ) <sub>2</sub>	MgSO <sub>4</sub>	CaCl <sub>2</sub>	CaCO <sub>3</sub>	TOTAL SALT CONCENTRATION
			<i>atm.</i>						
I	1	4.55	0.89	0.3715	0.2216	1.9261	.....	.....	2.5192
	2 (check)	4.54	1.00	0.3715	0.4430	1.9261	.....	.....	2.7406
	3	4.57	1.19	0.3715	0.8864	1.9261	.....	.....	3.1830
	4	4.58	1.57	0.3715	1.7728	1.9261	.....	.....	4.0694
	5	4.56	1.95	0.3715	2.658	1.9261	.....	.....	4.9558
II	6	4.48	1.18	0.3715	0.4430	1.9261	0.25	.....	2.9906
	7	4.47	1.35	0.3715	0.4430	1.9261	0.50	.....	3.2406
	8	4.39	1.68	0.3715	0.4430	1.9261	1.00	.....	3.7406
III	9	6.12	1.00	0.3715	0.4430	1.9261	.....	0.0420	2.7826
	10	4.50	.....	.....	.....	.....	.....	.....	0.3120
	11	6.28	.....	.....	.....	.....	.....	0.0375	0.3265

pared in order to avoid variations which may be brought about by some abnormalities in plant metabolism. Consequently, some preliminary work was carried out to test the effect on the growth of plants, of the culture solutions which were to be employed in this experiment.

The results of these preliminary tests showed that when calcium in the form of Ca(NO<sub>3</sub>)<sub>2</sub> was present in solution in concentrations of 27 mgm. or less per liter, the plants grew poorly, showing chlorosis and hypertrophy near the base of the stem. On the other hand, when the amount of this salt in solution was increased to 864 mgm. or more of Ca per liter the plants exhibited considerable etiolation. Culture solutions containing 450 mgm. of Ca as CaCl<sub>2</sub> per liter produced plants with large leaf blades having a thin, glossy epidermis. Later the leaves became mottled with symptoms somewhat resembling those usually

observed in plants suffering from the mosaic diseases. On the basis of these results, the culture solutions were made up of such concentrations as to include the minimum and maximum amounts of calcium and nitrogen that proved efficient in producing normal plants.

Group I consisted of five cultures in which the calcium and nitrogen concentrations were varied in equal proportions by increasing or decreasing the amount of  $\text{Ca}(\text{NO}_3)_2$  in the solution. Culture 2 was used as check, and cultures 1, 3, 4, and 5, contained one-half, two, four, and six times as much  $\text{Ca}(\text{NO}_3)_2$  as did the check solution. The composition of the culture solutions, their osmotic concentrations, and their pH values are given in table 1.

In the cultures from group II, the concentration of nitrogen was exactly the same as that present in the check, but the calcium was increased to 198 mgm., 288 mgm., and 468 mgm. per liter in solutions 6, 7, and 8, respectively, by the addition of different amounts of  $\text{CaCl}_2$ . The osmotic concentration values of the culture solutions in the first two groups ranged from 0.89 to 1.94 atmospheres for the lowest and highest salt concentrations respectively. The distilled water employed for all the culture solutions was obtained from a Barnsted still.

The third group consisted of cultures, 9, 10, 11. Culture 9 had the same composition as that of the check with the exception that the distilled water used to make up this solution had been previously saturated with  $\text{CO}_2$  and  $\text{CaCO}_3$ . The carbon dioxide was used to increase the solubility of calcium carbonate in solution. Cultures 10 and 11 consisted of soil extracts, the latter containing  $\text{CaCO}_3$ . Out of all the processes now in vogue for obtaining a soil solution (5, 6, 7, 14, 15, 34, 49, 52, 53), the water extraction method was chosen. The procedure in obtaining the extract was the same as that employed by the Bureau of Soils (45), except that the time of shaking, was 10 minutes instead of 3 minutes. The soil employed was a comparatively fertile Sassafras loam. Some difficulties were at first encountered in obtaining an extract free from colloidal material. The extracted solution remained turbid even after it was passed through a Pasteur-Chamberlain filter. It was, however, later discovered that a clear extract could be easily obtained when the pH of the soil (which was originally 4.92) was reduced to about 4.50. Evidently this pH corresponded to the isoelectric point of the soil colloids.

It was found by preliminary tests that by mixing the Sassafras loam with another soil which was still more acid, having a pH of 3.41, a mixture could be obtained, the reaction of which varied according to the proportions of the two soils used. Thus a mixture of one part of the very acid soil to eight parts of the Sassafras loam gave a water extract that ranged in pH from 4.46 to 4.54 as determined electrometrically from several extractions. This method was found more convenient and by far less time consuming than any one of the other methods tried in adjusting the reaction of the soil extract. The entire method briefly, consisted in shaking the mixture of the two soils for 10 minutes in five parts of water. The mixture was allowed to settle and the supernatant liquid filtered through a double filter paper.

The soil extracts thus obtained were comparatively rich in calcium and very poor in nitrogen. Four duplicate analyses of the extracts prepared at different times gave an average of 121 mgm. of calcium, 13 mgm. of magnesium, and only 7 mgm. of nitrogen per liter. For culture 11 the soil extract was first saturated with  $\text{CaCO}_3$  and again filtered before it was transferred to the jars.

The initial H-ion concentrations were approximately the same for all the solutions except for those containing  $\text{CaCO}_3$ . The pH values, as shown in table 1, ranged from 4.39 in culture 8, to 4.58 in culture 4, as compared with 4.54 for the check solution. The  $\text{CaCO}_3$  treatment lowered appreciably the H-ion concentration in the culture solution and in the soil extract, and the pH values for cultures 9 and 11 were 6.12 and 6.28 respectively.

The plants from group IV were grown in large galvanized iron tanks containing 100 pounds of soil. Mineral fertilizers in the form of 14 gm. of acid phosphate, 7 gm. of KCl, and 7 gm. of  $\text{NaNO}_3$ , were added to each tank, while  $\text{CaCO}_3$  was added to two of the tanks only. The soil used here was the same as that from which the extracts were obtained for the cultures of the previous group. The addition of 136 gm. of  $\text{CaCO}_3$  to each of the two tanks raised the pH of the soil from its original value of 4.92 to 6.70. The tanks were kept in the greenhouse and were watered frequently in order to maintain the moisture at about 50 per cent of the moisture holding capacity of the soil. Seeds taken from the same stock and of similar weight as those used for the solution cultures were planted on May 20. Twenty healthy seedlings were selected for each tank and were allowed to grow to maturity. The tops were harvested on August 22 and analyzed for the same constituents as were the plants from the solutions. In view of the fact that a large number of plants (forty) were harvested from the duplicate tanks for each treatment, it was not considered essential to repeat this part of the experiment as was done with the first three groups of plants.

The tops and roots were harvested separately from each culture and dried to constant weight at a temperature of  $65^\circ\text{C}$ . All the plant material from the corresponding cultures in each series was then combined, finely ground, and stored in tightly covered glass jars for chemical analysis. All the analyses were carried out in duplicate according to the Official Methods of the Association of Agricultural Chemists (40), and averages of the two determinations are presented in the tables. The calcium and magnesium were determined gravimetrically from the ash of the plants, while the protein was precipitated from the ground plant material and the nitrogen in the residue determined. For the total nitrogen determination the Kjeldahl method, modified to include nitrate nitrogen, was used.

## EXPERIMENTAL RESULTS

*Introductory.*

The results secured from each of the four groups of plants are presented in the succeeding tables separately and are then summarily discussed. The two different series of plants are designated *A* and *B* for the sake of clearness, and only one series of numbers (1 to 16) was employed to denote all the cultures of the different groups. The dry weights of tops and roots from the solution plants are given separately in tables 2, 4, and 6, and the chemical analyses of the same plants are shown in tables 3, 5, and 7. The data in table 8 represent both the dry weight and the composition of the tops from the soil plants. The appearance of the plants from groups I, II and IV are shown in plates 1, 2, and 3, respectively.

TABLE 2

*Dry weights of plants grown in culture solutions of group I containing varying concentrations of calcium nitrate*

CULTURE SOLUTIONS				DRY WEIGHTS								
Culture number	Milligrams per liter			Series A			Series B			Averages of Series A and B		
	Ca(NO <sub>3</sub> ) <sub>2</sub>	Ca	N	Roots	Tops	Total*	Roots	Tops	Total*	Roots	Tops	Total
1	222	54	38	4.46	41.65	46.11	3.08	14.80	17.88	3.77	28.33	31.50
2 (check)	443	108	76	4.99	59.21	64.20	3.80	22.85	26.65	4.39	41.03	45.43
3	886	216	152	6.08	58.27	64.35	4.30	32.00	36.30	5.19	45.14	50.33
4	1,173	432	304	9.38	73.74	83.12	6.30	40.90	47.50	7.84	57.32	65.31
5	2,658	648	456	7.94	73.72	81.86	5.00	35.50	40.50	6.47	50.46	61.18

\* Six plants.

*Group I*

An examination of the dry weights of the plants in group I (table 2) shows higher yields with increasing amounts of Ca(NO<sub>3</sub>)<sub>2</sub> until the calcium concentration reaches 432 mgm. per liter of solution. With higher concentrations of this salt a decided decrease in the dry weights of roots in both series and in the dry weights of tops only in series B is noticed. Thus, solution 4 gave the highest total yield in both series, while the very luxuriant growth occurred in plants from both solutions 4 and 5, where the Ca(NO<sub>3</sub>)<sub>2</sub> concentrations were highest, as may be clearly seen from plate 1.

The data from the chemical analyses presented in table 3 show a direct relation between the calcium content of the plants and the concentration of the same element in the solutions. The calcium content ranged from 0.55 per cent to 2.41 per cent in plants from the different cultures in series A and from 0.45 per cent to 2.44 per cent in those from series B. A clear generalization is here indicated. In each case a high calcium content in the plants corresponded to

a high concentration of this element in the culture solution, and a low calcium content in the plants corresponded to a low content of this element in the culture solution. This conclusion is perfectly definite for the conditions of this experiment, at least. On the other hand no such correlation was observed between the nitrogen content of the plants, either as total or as protein nitrogen, and the nitrogen content of the media. Although differences in the amount of nitrogen absorbed by the plants of the different cultures were observed, they were very slight and did not always correspond to the order of differences in the concentrations of this element in the culture solutions, and may not, therefore, be considered to have any significance in this connection. It is important to observe further that the same lack of relationship existing between the nitrogen content of the media and that of the plant is shown also for the calcium content of the media and the nitrogen content of the plants, since these

TABLE 3

*Composition of plants grown in culture solutions of group I, containing varying concentrations of calcium nitrate*

CULTURE SOLUTION				COMPOSITION OF PLANTS											
Culture number	Milligrams per liter			Series A				Series B				Averages of Series A and B			
	Ca(NO <sub>3</sub> ) <sub>2</sub>	Ca	N	Ca	Mg	Total N	Protein N	Ca	Mg	Total N	Protein N	Ca	Mg	Total N	Protein N
				per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
1	222	54	38	0.55	0.94	3.52	2.45	0.45	0.94	3.38	2.24	0.50	0.94	3.45	2.35
2 (check)	443	108	76	0.65	0.95	3.56	2.57	0.61	0.86	3.24	2.25	0.63	0.91	3.40	2.41
3	886	216	152	0.76	0.83	3.35	2.28	1.01	0.84	3.51	2.29	0.89	0.84	3.43	2.29
4	1,773	432	304	1.92	0.75	3.32	2.21	1.53	0.81	3.61	2.29	1.73	0.78	3.47	2.25
5	2,658	648	456	2.41	0.94	3.45	2.22	2.44	0.70	3.65	2.32	2.42	0.82	3.50	2.27

two elements varied in the culture solutions of this group in the same proportions. That is, variation in the calcium and nitrogen content of the culture media had no influence whatever on either the total nitrogen or the protein nitrogen of the plants. This is in direct variance with the definite relationship shown between calcium content of medium and of plant.

Comparing now the calcium and magnesium contents of plants and media it becomes obvious that a slight decrease of the latter occurred with considerable increase in the former. This relationship is quite definite for the culture of series B, but is not so definitely obvious for those of series A. Considering averages of the two series, however, the calcium content of the plants of the several cultures shows increasing percentage values ranging from 0.50 to 2.43 whereas magnesium shows a corresponding decrease in percentage values ranging from 0.94 to 0.78.

*Group II*

The cultures in group II differed from those in the previous one only in the source of calcium used;  $\text{CaCl}_2$  instead of  $\text{Ca}(\text{NO}_3)_2$ , in successive increments of 0.25 gm., 0.50 gm., and 1 gm. per liter of solution was added to cultures 6, 7, and 8 respectively. Consequently the amount of nitrogen in each of the three cultures in this group remained unchanged from that initially present in the check solution; namely, 76 mgm. per liter.

TABLE 4

*Dry weight of plants grown in culture solutions of group II treated with calcium chloride*

CULTURE SOLUTIONS				DRY WEIGHTS								
Culture number	Milligrams per liter			Series A			Series B			Averages of Series A and B		
	$\text{CaCl}_2$ added	Ca	N	Roots	Tops	Total	Roots	Tops	Total	Roots	Tops	Total
6	250	198	76	5.38	44.92	50.31	4.50	27.00	31.50	4.94	35.96	40.90
7	500	288	76	5.15	51.34	56.49	4.95	42.10	47.05	5.05	46.72	51.77
8	1,000	468	76	6.64	51.94	58.58	5.30	44.70	50.00	5.97	48.32	54.29
Check	None	108	76	4.99	59.21	64.20	3.80	22.85	26.65	4.39	41.03	45.43

TABLE 5

*Composition of plants grown in culture solutions of group II treated with calcium chloride*

CULTURE NUMBER	CULTURE SOLUTIONS			COMPOSITION OF PLANTS											
	Milligrams per liter			Series A				Series B				Average of Series A and B			
	$\text{CaCl}_2$ added	Ca	N	Ca	Mg	Total N	Protein N	Ca	Mg	Total N	Protein N	Ca	Mg	Total N	Protein N
6	250	198	76	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
7	500	288	76	1.27	0.85	3.48	2.49	1.29	1.04	3.36	2.27	1.28	0.95	3.42	2.38
8	1,000	468	76	1.39	0.80	3.51	2.31	1.33	0.78	3.27	2.20	1.36	0.79	3.38	2.26
Check	None	108	76	1.94	0.87	3.32	2.25	2.05	0.72	3.55	2.40	2.00	0.79	3.43	2.33
				0.65	0.95	3.56	2.57	0.61	0.86	3.24	2.25	0.63	0.91	3.40	2.41

In general the addition of  $\text{CaCl}_2$  in the concentrations enumerated above did not exert any injurious influence on the growth of the plants. They appeared normal in every respect and produced good pods, as may be seen from plate 2 (showing photographs of the plants from series B) as well as from the dry weights given in table 4. Considering averages of the dry weights of tops from the two series, no marked benefits were derived from the addition of increasing increments of  $\text{CaCl}_2$  to the culture solution. On the other hand, the dry weights of the roots were considerably higher in each of the three cultures than were those harvested from the check solutions, thus exhibiting



again, as has already been shown by the results from group I, the beneficial influence of the calcium addition on root development.

The data from the chemical analyses, given in table 5, show again a direct relation between the concentration of calcium in solution and the amount of this element absorbed by the plants, the relation being the same as that found in the plants of the preceding group. Plants from both series increased their calcium content with increasing amounts of  $\text{CaCl}_2$  in the solution.

The percentages of the nitrogen varied somewhat, as is to be expected, but there is no correlation between nitrogen and the calcium content of the plants. This lack of correlation was also indicated for the plants of group I. Slight differences also occur in the protein-nitrogen content, but these differences are not significant and the protein-nitrogen appears to have no direct relation to either the calcium in the culture solution or in the plant.

TABLE 6

*Dry weights of plants grown in culture solutions of group III and in soil extracts treated with calcium carbonate*

CULTURE NUMBER	CULTURE SOLUTION			DRY WEIGHTS								
	Milligrams per liter			Series A			Series B			Average of Series A and B		
	$\text{CaCO}_3$ added	Ca	N	Roots	Tops	Total	Roots	Tops	Total	Roots	Tops	Total
9	42	1.25	76	4.94	44.67	49.61	2.85	23.10	25.95	3.89	33.84	37.78
10*	.....	1.21	7	7.08	37.08	44.16	.....	.....	.....	.....	.....	.....
11†	37	1.35	7	8.90	38.24	47.14	.....	.....	.....	.....	.....	.....
Check	None	1.08	76	4.99	59.21	64.20	3.80	22.85	26.65	4.39	41.03	45.43

\* Soil extract.

† Soil extract and  $\text{CaCO}_3$ .

Appreciable differences in the magnesium content were observed in plants from series B, the percentage values of this element in the plants decreasing progressively with increasing values for calcium. Considering averages of the two series, this relation is more definitely shown for the cultures of this group than it is for the cultures of group I.

It may be of interest to note here that the plants grown in solutions containing high concentrations of  $\text{CaCl}_2$  or  $\text{Ca}(\text{NO}_3)_2$  notably cultures 4, 5, 7, 8, required considerably less iron to maintain the normal green color than did the plants grown in solutions low in concentrations of calcium.

### *Group III*

Group III includes cultures 9, 10, 11. Culture 9 had exactly the same concentration of the three initial salts as had the check solution with the exception that the former was saturated with  $\text{CaCO}_3$ . Culture 10 represents the untreated soil extract, and culture 11 consists of the same soil extract treated with

$\text{CaCO}_3$ . The numerical data concerning the dry weights and the chemical analyses of the plants of this group are given in tables 6 and 7.

The plants from culture 9 show an average total yield somewhat less than that from the check culture, but a marked increase is observed in the nitrogen content of these plants over those of the checks. The data of the chemical analyses show that the total nitrogen percentage (3.93 for series A and 3.81 for series B) for the two series was higher in these plants than in the plants from any of the cultures in the preceding groups. It is important to note that this superiority in nitrogen content is not manifested in the protein nitrogen of the plants. The percentage values of protein nitrogen found in the plants of the two series in question are not higher than those shown for the check plants or for the plants of any of the cultures of the preceding groups. The higher

TABLE 7

*Composition of plants from group III grown in culture solutions and in soil extracts treated with calcium carbonate*

CULTURE NUMBER	CULTURE SOLUTIONS			COMPOSITION OF PLANTS											
	Milligrams per liter			Series A				Series B				Average of Series A and B			
	$\text{CaCO}_3$ added	Ca	N	Ca	Mg	Total N	Protein N	Ca	Mg	Total N	Protein N	Ca	Mg	Total N	Protein N
9	42	1.25	76	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
10*	.....	1.21	7	0.79	0.84	3.93	2.40	1.19	0.84	3.81	2.25	0.99	0.84	3.87	2.33
11†	37	1.35	7	1.02	0.46	1.51	1.42	.....	.....	.....	.....	.....	.....	.....	.....
Check	None	1.08	76	1.40	0.61	1.81	1.49	.....	.....	.....	.....	.....	.....	.....	.....
				0.65	0.95	3.57	2.57	0.61	0.86	3.24	2.25	0.63	0.91	3.40	2.41

\* Soil extract.

† Soil extract and  $\text{CaCO}_3$ .

amount of nonprotein nitrogen found in these plants may be due to the fact that the initial H-ion concentration of this solution was considerably lower than that of the other cultures considered. This fact will be further emphasized in the following sections. The initial H-ion concentration of the culture corresponded to a pH value of 6.12 as compared with a pH of 4.54 of the check solution.

In the soil extracts the seedlings grew poorly during the first three weeks and showed characteristics usually exhibited by plants suffering from lack of nitrogen. During the fourth week, however, they began to show more vigorous growth, produced good leaves, and grew to maturity. This sudden change in the behavior of these plants was accompanied by vigorous nodule formation.

It may be of interest to mention here that practically all the nodules were formed on the base of the roots, that is, either entirely above or close to the

surface of the solution. Whether this was due to the greater abundance of oxygen on the surface, or to some inhibiting factor exerted by the solution, is at present not clear. It seems evident, however, that the reaction of the solution had no effect upon the process of symbiotic nitrogen fixation. The nodules were just as abundant on roots grown in the solution of culture 10 with a pH of 4.50 as they were on the roots of culture 11, in which the calcium carbonate lowered the initial H-ion concentration to 6.28.

Although no further nitrogen starvation was observed, the plants grown in the soil extracts never completely regained the rich green color which was displayed by the plants grown in the culture solutions. They were pale green throughout the growth period. It must be remembered here that the soil extracts used were very poor in nitrogen, containing only 7 mgm. per liter of extract.

It is regrettable that this part of the experiment could not be successfully repeated in the second series, as no nodules formed and the plants starved from lack of nitrogen and died after five weeks of scanty growth. The failure of the plants to form nodules may be explained, possibly, by the fact that the soil from which the extracts were made was kept in the greenhouse exposed to sunlight and may have become free from the symbiotic nitrogen-fixing bacteria by the time the second series was conducted. Inoculating the soil extract with a fresh culture of *B. radicicola* (soybean variety) when the plants had already reached an advanced stage of nitrogen starvation did not stimulate the roots to nodulation. These plants were discarded and the results from cultures 10 and 11 in series B are, therefore, not presented in the tables.

A comparison of the yields of the plants from the two soil extracts shows considerably higher dry weights of roots and only slightly higher dry weights of tops in favor of the  $\text{CaCO}_3$  treatments. The application of  $\text{CaCO}_3$  also increased both the calcium and the magnesium in the plants. Those from the untreated culture contained 1.02 per cent calcium and 0.46 per cent magnesium, whereas the plants from the treated culture showed corresponding values of 1.40 and 0.61, respectively.

The nitrogen content of these plants was considerably lower than in those from the culture solutions. Evidently the amount of nitrogen assimilated from the air by the symbiotic process was not sufficient to supply the plants' requirement. The plants from the soil extract to which  $\text{CaCO}_3$  was added were appreciably higher in total nitrogen than were those from the untreated extract, but the percentage of protein nitrogen was approximately the same for both cultures. This higher total nitrogen content was here, as in culture 9, accompanied by a higher calcium content than is shown for the corresponding check cultures. Thus, in the two types of culture media here represented, the addition of calcium in the form of carbonate appreciably increased the total nitrogen of the plants but had no influence whatever on the protein nitrogen.

*Group IV*

Group IV, includes the plants from the limed and unlimed soils. Since duplicate tanks were used for each treatment, the dry weights, as well as the chemical analyses are given separately for each tank in table 8. The most significant influence of the  $\text{CaCO}_3$  observed in this group was in the amount of dry weight produced by the plants. The total crop from tanks 13 and 14 was 62.55 gm., whereas 96.40 gm. were harvested from tanks 15 and 16, which received lime. These differences are clearly brought out by the photographs in plate 3. This fact becomes more interesting because no such differences were found in the plants from the soil extracts in group III. The extract saturated with  $\text{CaCO}_3$  produced only 3 gm. of dry weight more than the untreated extract, as is shown in table 6. It is, therefore, reasonable to assume that the soil plants were much more benefited by the presence of  $\text{CaCO}_3$  than were the solution plants. This assumption is further strengthened by two

TABLE 8  
*Composition and dry weight of plants from group IV grown on limed and unlimed soil*

TANK NUM- BER	TREATMENT	SOIL pH	TOPS	TOTAL	Ca	AVER- AGE	Mg	AVER- AGE	TOTAL N	AVER- AGE	PRO- TEIN N	AVER- AGE
			gm.		per cent		per cent		per cent		per cent	
13	Minerals	4.92	33.30	62.55	1.59	1.53	0.40	0.43	3.31	3.30	2.32	2.36
14	Minerals	4.92	29.25		1.47		0.46		3.29		2.40	
15	Minerals and $\text{CaCO}_3$	6.70	48.80		2.71		0.34		3.59		2.40	
16	Minerals and $\text{CaCO}_3$	6.70	47.60	96.40	2.49	2.60	0.35	0.35	3.47	3.53	2.26	2.33

facts: First, that the plants from the limed tanks absorbed a considerably higher percentage of calcium than did those from the "limed" solutions, and secondly that they contained 70 per cent more of this element than did the unlimed plants, as may be seen from table 8.

It may also be of interest to note that the magnesium content in the soil plants was lower than in those from any one of the culture solutions and that the higher Ca content was accompanied by a slightly lower magnesium content. The average magnesium content of the unlimed plants was 0.43 per cent whereas that of limed plants was 0.35 per cent. The amounts of nitrogen and of protein found in these plants did not show any pronounced differences from those found in the plants grown in the solutions. Comparing, however, the total nitrogen of the plants from the limed and unlimed soils, respectively, an increase of 0.23 per cent is observed with the application of  $\text{CaCO}_3$ . On the other hand, the average protein-nitrogen content of the plants from the two respective soils remained practically the same.

In general, the soil plants and the solution plants did not show any striking differences in composition. The application of  $\text{CaCO}_3$ , irrespective of the medium employed, produced similar effects on the growth of the plants. In each case where this salt was used there occurred a marked increase in the calcium, a considerable increase in the nitrogen, and no appreciable change in the protein nitrogen content of the plants. But so far as the total yield was concerned, the influence of  $\text{CaCO}_3$  was strongly marked in the soil plants and little in the solution plants.

Finally, attention must be called to the fact that the total yield from series B was lower than that from series A. Since this phenomenon took place in all the culture solutions, it may be ascribed to the difference in the season. During the summer months when series B was carried out, the plants matured nearly two weeks earlier and did not make such a vigorous growth as did the plants from series A grown during the spring months.

#### DISCUSSION

A general survey of the average results obtained from the four groups of cultures brings out several interesting correlations. The average percentage values of the calcium content of the plants grown in culture solutions, in soil extract, or in soil, always follow the order of concentrations of this element in the medium, regardless of the calcium salt used and independently of the amount of nitrogen present. It appears, however, that the plants absorbed calcium more readily from  $\text{CaCO}_3$  than from equivalent concentrations of this element in the form of  $\text{Ca}(\text{NO}_3)_2$  or  $\text{CaCl}_2$ . The plants showed no tendency to absorb more calcium from  $\text{Ca}(\text{NO}_3)_2$  than from  $\text{CaCl}_2$  when present in the medium in equivalent concentrations of calcium, as was observed by Reed and Haas (43) in the case of citrus seedling.

On the other hand, the nitrogen content of the substratum appears to have no definite relation whatever to the nitrogen content of the plants, provided this element is present in the medium in sufficient concentration to supply the needs of the plants. Neither was the nitrogen content in the plants influenced by the concentration of calcium in the medium present in the form of  $\text{Ca}(\text{NO}_3)_2$  or  $\text{CaCl}_2$ . In the presence of  $\text{CaCO}_3$ , however, the plants showed an appreciably higher total nitrogen content than in the presence of equivalent concentrations of calcium in the form of nitrate or chloride in the medium, irrespective of type; but in each instance this increased total nitrogen content of the plants was accompanied by a relatively much lower H-ion concentration of the medium than that of those in which the plants show no appreciable change in total nitrogen content with considerable variation in the calcium concentration of the media. This suggests that any superiority in the total nitrogen content of the plants over that of the checks is determined by the reaction and not by the calcium content of the solution, since higher increments of Ca in the medium had no influence upon the total nitrogen content of the plants unless accompanied by a corresponding decrease in the H-ion concentration.

The results do not offer a single example which might indicate a definite calcium-protein relationship in the soybean plants. In general the protein-nitrogen percentages ran considerably lower in all plants (except those grown in the soil in which nitrogen was deficient) than did the total nitrogen percentages, fluctuating between 2.25 per cent and 2.38 per cent. The protein content remained approximately constant and was independent of either the calcium or the total nitrogen content in the plants or in the media, except where the nitrogen supply in the media was insufficient for the requirements of the plants, as in the case of the soil extracts. These results lead to the assumption that the excess of nitrogen observed in the soybean plants grown on limed soil over that of the plants grown on unlimed soil is present only in the nonprotein form.

Thus the results secured from the present experiments emphasize strongly the fact that no direct relationship exists between the calcium and protein metabolism in the soybean plants. On the other hand it appears that higher quantities of total nitrogen were absorbed by the plants when grown in solutions containing  $\text{CaCO}_3$  than in solutions where this salt was absent. It appears further, that both the calcium and nitrogen content of the plants increased when the pH of the solution was brought up to 6.0 or slightly higher by the addition of calcium carbonate.

#### SUMMARY AND CONCLUSIONS

Soybean plants grown to maturity on limed and unlimed soil, in soil extracts saturated with calcium carbonate, and in complete culture solutions containing varying concentrations of calcium and nitrogen, were analyzed for protein nitrogen, total nitrogen, calcium, and magnesium. Calcium chloride, calcium carbonate, and calcium nitrate were used in order to obtain different calcium-nitrogen ratios without otherwise disturbing the nutrient balance of the culture solutions.

1. A definite correlation between the amount of calcium in the culture solution and the calcium content of the plant was observed. The percentage of calcium in the plants increased with the increase in the concentration of this element in the culture solutions.

2. No definite correlation was observed between the amounts of nitrogen in the medium and in the plants. Increasing the nitrogen concentration of the culture solution did not appreciably alter the nitrogen content in the crop. Neither was the nitrogen content of the plants influenced by the calcium when calcium chloride or calcium nitrate was used; but a higher content of both nitrogen and calcium occurred in the plants in the presence of calcium carbonate than in the presence of either calcium nitrate or calcium chloride, irrespective of the medium employed.

3. High total nitrogen in the plants was definitely correlated with low H-ion concentrations in the medium.

4. No relationship was found between the calcium and the protein content in the soybean plants.

5. The plants showed higher rates of nitrogen absorption in the presence of  $\text{CaCO}_3$  than in the presence of the other Ca compounds employed, but the increased rates of nitrogen absorption did not at all influence the protein content of the plants.

6. Culture solutions containing high concentrations of either calcium nitrate or calcium chloride produced plants which required less iron than did the plants grown in the solutions containing calcium carbonate.

7. In general, the soil plants did not show any striking differences in their composition from the plants grown in culture solutions.  $\text{CaCO}_3$  had little or no accelerating influence upon the growth of the plants in the solution cultures, but had a marked accelerating effect upon the growth of the plants in the soil cultures.

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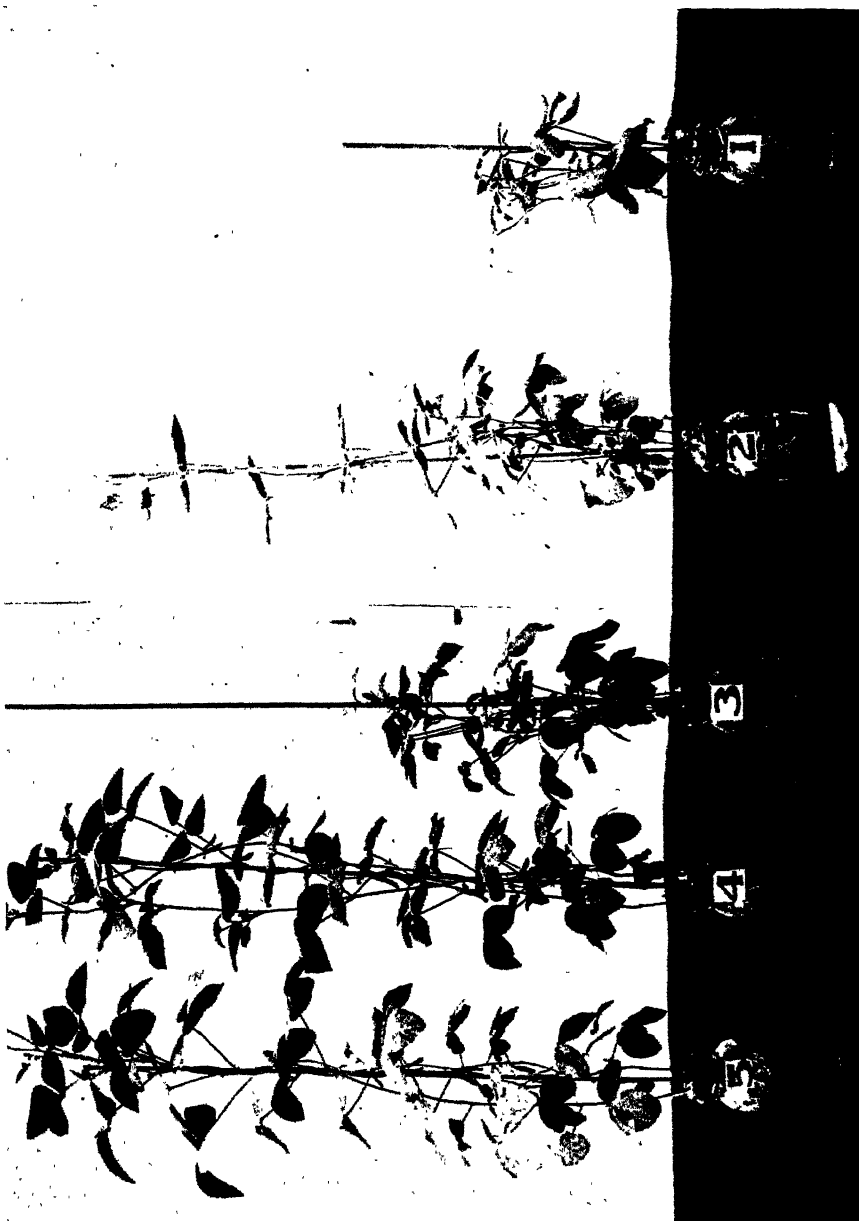
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## PLATE 1

GROUP I. PLANTS FROM SERIES B GROWN IN CULTURE SOLUTIONS (1, 2, 3, 4, 5) CONTAINING VARYING CONCENTRATIONS OF CALCIUM NITRATE



## PLATE 2

GROUP II. PLANTS FROM SERIES B GROWN IN CULTURE SOLUTIONS (6, 7, 8) CONTAINING  
VARYING CONCENTRATIONS OF CALCIUM CHLORIDE



## PLATE 3

GROUP IV. PLANTS GROWN IN LIMED (TANK 15) AND UNLIMED (TANK 13) SOILS





# SOME OBSERVATIONS UPON THE EFFECT OF THE SIZE OF THE CONTAINER UPON THE CAPILLARY RISE OF WATER THROUGH SOIL COLUMNS<sup>1</sup>

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Although numerous records of the capillary rise of water through soil masses are to be found in agricultural literature, there is little reference made to the cross-sectional area of the soil column under consideration. It is clear that most workers in this field have assumed that the size of the column is a factor of little or no importance. Keen (3) gives a mathematical formula for the ultimate capillary rise of water through soil masses in which the average size of soil particles is known, but no factor in his formula indicates the effect of varying sizes of columns which might be put under observation. Hilgard (2) and other writers give the maximum capillary rise obtained in soils of varying properties, without mentioning the cross-sectional area of the columns used. Rotmistrov (6) working in the Odessa Experimental Field reports that a greater rise of water by capillarity was noted in small columns than in large columns when each size was filled with soil of similar texture. Risler and Wery (5) state that capillary rise is more rapid when large tubes are used for experimental observations than when small tubes are used, but no experimental evidence is offered to support the statement. No reference is made to the total rise experienced under these conditions.

The increasing use of tanks as a means of studying the use of water by plants and in observations upon the effect of a high water table upon plant growth prompted a careful study of the effect of the size of the container upon the capillary rise of water through soils.

## PRELIMINARY OBSERVATIONS

Preliminary observations with the assistance of R. E. Storie<sup>2</sup> were made upon four cylindrical columns of sheet celluloid with exterior reinforcement of quarter-inch wire screen. These columns were filled with screened, well mixed, air-dried soil, uniformly packed and equal in volume weight. These columns were erected in a large constant temperature chamber, which was equipped with an electric heating element and a thermostat and maintained

<sup>1</sup>Published with the permission of the Director of the Experiment Station.

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at 85°F. A thermograph placed in the constant temperature chamber showed a maximum variation of 1.5°F. during the period of observation.

The columns were erected over a galvanized iron base pan in which the pre-determined water level was maintained by a simple water stage regulator. The columns were submerged to a depth of 2 inches. Observations upon capillary rise through these several columns were made daily until the slowness of rate made less frequent observations acceptable. In all cases the magnitude of capillary rise as shown in table 1 measures the distance in centimeters from the constant water level to the point of capillary rise after the time interval noted. Table 1 gives a summary of the results obtained together with details of column sizes and the initial volume weights.

TABLE 1

*Details of preliminary observations upon capillary rise through columns of varying cross sections*

	COLUMN 1	COLUMN 2	COLUMN 3	COLUMN 4
Mean inside diameter, inches.....	1	1 $\frac{1}{8}$	3 $\frac{1}{2}$	5 $\frac{1}{2}$
Initial volume weight, gm.....	1.41	1.39	1.48	1.39
Discrepancy of volume weight from mean, per cent.....	-0.07	-2.11	+4.22	-2.11

DAYS	OBSERVED RISE			
	cm.	cm.	cm.	cm.
1	53.3	54.9	54.3	62.0
5	61.1	64.8	64.8	69.6
10	64.0	69.4	70.8	73.5
20	67.0	73.1	75.0	81.0
41	73.4	86.7	89.5	101.2
63	78.4	92.7	96.4	107.9

#### DETAIL OF APPARATUS

Although these results may be considered as indicative of a tendency, they are insufficient as a basis for a general statement with regard to the effect of the size of the container upon the capillary rise of water through soil masses. A second and more comprehensive installation was designed in October, 1924, to furnish more evidence.

In the new installation cylindrical columns were abandoned because of the difficulty of finding a suitable material for use in the larger sizes. Sheet cel-luloid has an unfortunate property of buckling upon handling which makes it unsuitable for use in cylinders larger than six inches in diameter. Glass in large sizes was considered as economically impractical. Square wooden columns, somewhat similar to those used by McLaughlin (4), except that the galvanized iron lining was omitted, were finally decided upon. Redwood lumber was used exclusively in the construction of the boxes. The unsurfaced faces of the boards were set inside the boxes in all cases. Backs and sides

were milled to fit exactly. Prescribed dimensions were maintained within the limits of good mill practice. Backs and sides were rabbeted in place and fastened with screws. Channels were milled on the side boards to receive plates of 26-ounce window glass which was cut to fit. Suitable cleats held the glass in place. In every case the bottom of the soil chamber was 2 inches above the bottom of the column. Bottoms were formed of pieces of perforated galvanized iron supported on cleats screwed to the sides of the columns. Fine screen and muslin cloth placed over this perforated plate prevented the soil from sifting through but at the same time gave ready entrance to water. All columns were painted on the inside with light asphalt paint to minimize the absorption of water by the wood. All columns were 5 feet 2 inches long, providing a net length of soil column of 5 feet.

All columns were square in cross section. The following sizes were used: 1 inch, 2 inches, 3 inches, 4 inches, 5 inches, 6 inches, 8 inches, and 12 inches square. Each size group contained four individuals in order that differences in rise of capillary water might logically be attributed to the size of the column and not to some individuality of a single column. Columns were so numbered that the last digit indicated the number of the individual within the size group. The first digit or the first two indicated the size group. This numbering will be adhered to in the following discussion. The columns were installed vertically in four galvanized iron base pans prepared for the purpose. Gage glasses were fixed to each of the pans so that the water level might readily be observed. A fine line on the gage glasses indicated the proper depth for a 2-inch submergence of all the columns in the tank. Constant water level devices were arranged for each of the tanks so that the predetermined water level might be maintained with a minimum of attendance. Distilled water was used throughout the course of the observations.

One individual of each size group was established in a separate base pan for observations on the rate of water absorption. Water-tight partitions divided this base pan into separate compartments in which the columns were erected. Individual water stage regulators, provided with calibrated reservoirs were established on each of these small compartments. Losses by evaporation from the surface of the water were minimized by lids of light galvanized iron carefully fitted over the tops of the individual compartments. It was discovered after the start of the capillary rise that the constant water level devices were not sufficiently accurate for determining the amount of water used by the several columns. This was especially true of the smaller columns which were mounted in relatively large tanks.

#### THE SOIL USED

The soil used was from the farm of the Branch College of Agriculture at Davis, California and is classified as Yolo fine sandy loam. It is a recent alluvial soil derived from sedimentary rocks. This series is representative of large scattered areas in California. The soil was carefully screened through a

2-mm. mesh, the coarser material being rejected. After screening, the soil was shoveled six times on a concrete floor to insure thorough mixing. A composite of random samples of the screened and mixed soil mass showed the mechanical analysis according to the method of the Bureau of Soils, given in table 2.

TABLE 2  
*Mechanical analysis of soil used in capillary rise columns*  
Bureau of Soils Method

GRADE	MECHANICAL ANALYSIS	
	mm.	per cent
Fine gravel.....	2-1	0
Coarse sand.....	1-0.5	0
Medium sand.....	0.5 -0.25	0.622
Fine sand.....	0.25-0.10	23.152
Very fine sand.....	0.10-0.05	54.866
Silt.....	0.05-0.005	13.326
Clay.....	Less than 0.005	8.710

TABLE 3  
*Weights of soil mass in capillary columns*  
Pounds per cubic foot

INDIVIDUAL IN SIZE GROUP	SIZE GROUP IN INCHES							
	1	2	3	4	5	6	8	12
1	81.82	83.07	82.12	82.84	82.86	82.27	82.27	80.18
2	85.51	81.82	81.85	82.80	82.69	81.89	81.85	81.89
3	85.32	82.81	82.43	82.39	83.41	82.54	82.50	81.02
4	84.67	82.92	83.35	82.69	82.69	81.82	83.26	80.18
Average.....	84.33	82.65	82.44	82.68	82.91	82.13	82.47	80.82
Average apparent density in pounds per cubic foot.....	1.35	1.33	1.32	1.33	1.33	1.32	1.32	1.30

The moisture equivalent for the soil in question was determined in accordance with the method indicated by Veihmeyer, Israelsen and Conrad (7). Four determinations were made, the average moisture equivalent being 16.64 per cent.

#### FILLING THE SOIL COLUMNS

Every care was used to provide uniform densities of soil masses within the several columns during the operation of packing. After a number of trials, the following method of filling was devised. A section of 2-inch leader pipe,

5 feet long, was lowered into the column to be filled. The leader pipe was then filled with the mixed soil by means of a funnel and scoop. As the leader pipe was lifted from the bottom of the column, soil was discharged. The soil was uniformly distributed over the bottom of the column by moving the pipe. It was found that the collection of larger granules against the glass face of the column could be lessened by keeping the soil mass slightly higher against the glass face than near the back of the box. Where an accumulation of larger particles behind the glass persisted, a piece of heavy iron wire flattened at one end was inserted between the soil mass and the glass. By manipulation of this wire a uniform soil mass was created behind the glass. Figure 1 shows the procedure of filling the soil columns. The irregularities in color on the faces of the columns already packed, as shown in this figure, are due to shadows. A flash light was used in making the exposure.

The soil used in filling each column was carefully weighed on a triple beam balance weighing to one gram. An 8-inch column was filled first. During the operation of filling, light uniform hammer blows on the back and sides of the column aided in securing uniform density throughout the soil mass. When the column was filled, the average density of the soil mass within the column was obtained by dividing the weight of equivalent oven-dry soil used in filling the column by the volume occupied by the soil mass. Other columns were filled upon the basis obtained from the first one filled. From a consideration of the relative volumes to be occupied by the several columns, the amount of soil necessary to bring about the required density could be readily determined in each case. Some columns were packed several times before an acceptable density was obtained. An apparent specific gravity of 1.3 is about what is found by observations for volume weight on this soil type under field conditions.

Table 3 gives the results obtained in these efforts to secure uniform packing.

#### METHOD OF OBSERVATION

Base pans were filled to the predetermined level at as near the same moment as possible. Not more than seven minutes elapsed between the filling of the first pan and the completion of the operation.

Observations on the capillary rise in the several columns were made by noting the position of the line separating the dry soil from the wetted soil. Recorded observations measured in centimeters the position of this line above the plane of the water level. Fine nails driven in the cleats covering the glass furnished convenient datum points for measurement. During the early course of the observation the line of demarcation between dry soil and wetted soil was extremely distinct, making observations easy and accurate. Toward the end of the run a feathering out was noted which presumably decreased the accuracy of the reported observations. This condition was especially true with the smaller sizes which showed no real increase in capillary rise near the end of the period of observation. Irregularities in the line across the glassed faces of the larger columns made accurate observations difficult. In cases where consider-

able irregularity (never more than 5 cm.) in the line of demarcation occurred, a large celluloid triangle was used to obtain the point of average rise. Observations were made by the same individual insofar as was possible during the entire period of rise in order that personal error might be reduced and compensated.

Observations were made at the same hour each day. During the initial period of the rise, observations were made daily. As the rate of rise became slower the time interval between readings became longer. The apparatus was visited daily to provide necessary distilled water and to adjust the constant

TABLE 4

*Average rise of capillary moisture as observed in columns of varying cross-sectional areas after time intervals as noted*

(Each value is the average of measurements of four columns)

DAYS RUN	SIZES OF SQUARE COLUMNS AS MEASURED BY ONE DIMENSION IN INCHES							
	1	2	3	4	5	6	8	12
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
1	53.3	53.8	57.3	59.1	59.0	59.1	58.3	58.6
5	70.8	74.3	78.9	82.3	81.9	82.8	82.6	83.6
10	75.8	81.2	87.1	92.1	90.7	92.7	91.9	93.6
20	78.3	86.5	95.0	100.3	99.7	102.2	101.1	103.6
40	81.0	92.8	105.1	110.7	110.2	112.1	111.7	114.7
61	83.3	97.9	112.4	116.7	117.3	118.8	118.7	121.5
82	84.8	105.7	118.8	121.6	123.2	124.4	124.4	127.3
102	85.6	111.8	123.4	125.1	127.4	129.0	128.7	131.3
122	85.6	114.9	126.8	128.1	130.8	132.1	131.9	134.5
138	85.6	116.5	129.4	129.7	133.4	134.8	134.4	137.0
151	85.6	117.5	130.9	130.9	134.5	136.0	135.8	138.4
178	85.6	118.2	133.4	133.2	137.7	138.9	138.8	141.6
192	85.6	118.2	134.4	134.1	138.8	140.7	139.7	142.9
222	85.6	118.2	136.1	136.1	141.6	142.4	142.6	146.1
263	85.6	118.2	136.3	136.7	141.8	143.6	143.3	147.2

water level devices. The period of capillary rise extended from November 13, 1924 to August 4, 1925, or 263 days.

Figure 2 shows detail of installation and capillary rise in four of the smaller columns after sixteen days. The columns are 1 inch, 2 inches, 3 inches, and 4 inches square, reading from left to right.

#### SUMMARY OF OBSERVATIONS

The summary in table 4 shows the results of the observations made on the 32 capillary columns on different dates during the course of the rise. Detail with regard to individual columns is omitted for the sake of compactness, and the averages for the four columns making up the size group are recorded.

It is evident that the size of the column is a more important factor in determining the rise through capillary soil columns when the columns are relatively small than when the columns are large. In the four smallest groups every period of observation indicated a greater rise in the slowest column in one group than the highest rise in the next smaller group. The consistency of increase of rise with increase in size is reduced in the larger sizes. In fact the average rise for the 8-inch column for the time intervals noted was, in most cases, slightly less than for the 6-inch columns. A careful scrutiny of the detailed results of these observations indicate that a maximum difference of 11 cm. was recorded among the individuals of the same size group after the same time interval. The maximum differences due to the individuality of the columns were noted in the columns smaller than 25 square inches in cross-sectional area.

#### DISTRIBUTION OF MOISTURE

The moisture content present at different distances from the water table in each column was determined at the end of the experiment. The glass fronts of the columns were removed and the columns were tipped back slightly so that the top was about 2 inches from vertical. This was imperative in order that the dry soil near the tops of the columns might not fall out during the operation of sampling. A cheese sampler cutting a core  $\frac{5}{8}$  inch in diameter was used to obtain a section of soil from the front to the rear of each column at the various heights. As it was impossible to obtain samples closer to the water level than 3 inches because of the sides of the water reservoirs, the sampling was limited at the lower end of the columns to this height.

The samples were dried at 100°C. for 48 hours, cooled to original room temperature, and the moisture content calculated on the basis of the oven-dry weight. The results of these observations for moisture content seem to indicate that in columns larger than 9 square inches in cross-sectional area the same moisture content can be expected at points representing the same percentage of the total rise above the plane of the water table. In columns smaller than 9 square inches in cross-sectional area, slightly higher percentages of moisture were found at the same relative elevations. In general the differences between the moisture contents taken from points representing the same percentage of the total rise in all the columns under consideration were never more than 3 per cent. Results of these moisture determinations indicate that a slightly higher percentage of moisture is to be found in the soil mass a few inches above the plane of the free water table than immediately above it as was first reported by McLaughlin (4). In the smaller sizes, such as the 1-inch, 2-inch, and 3-inch columns, this zone of maximum moisture content was only from 3 to 6 inches above the plane of the water table. In the 12-inch columns the zone of maximum moisture content was found to be from 11 to 15 inches above the water table.

In view of the differences in total rise in individuals within the same size group, it is unsatisfactory to report the zone of maximum moisture content

for a given size group in terms of centimeters. Table 5 gives the location of the zone of maximum moisture content for each of the columns in terms of its percentage of the total rise. The average of the four columns is also given.

TABLE 5

*Location of zones of greatest moisture content in capillary columns of varying cross-sectional areas*

Distance of zone of maximum water content above the water table is given as a percentage of the total rise

INDIVIDUAL WITHIN SIZE GROUP	DISTANCE OF SQUARE COLUMNS AS PERCENTAGE OF TOTAL RISE TO WATER TABLE							
	1	2	3	4	5	6	8	12
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
1	8.3	12.2	16.2	27.3	27.2	20.9	19.9	20.7
2	15.8	11.0	19.0	22.9	21.2	26.9	21.3	20.8
3	9.3	11.0	20.3	18.2	16.0	19.4	17.6	20.7
4	14.2	15.2	22.9	22.7	18.2	26.2	26.2	20.7
Average. . .	11.7	12.4	19.6	22.8	20.7	23.5	21.3	20.7

TABLE 6

*Observations on the uniformity of distribution of moisture in samples taken from the same elevation above the constant water level, but at different points within the cross section—column 123*

LOCATION NUMBER	ELEVATION OF SURFACE SAMPLED, IN FEET ABOVE WATER LEVEL			
	1	2	3	4
1	33.2*	29.3	23.4	17.3
2	33.9	29.0	23.6	17.3
3	33.2	28.7	23.6	18.6
4	34.3	29.0	23.5	18.6
5	34.0	29.2	23.3	17.2
6	32.7	29.0	23.7	18.4
7	32.1	28.6	23.8	18.3
8	33.5	28.3	23.1	17.9
9	33.7	29.6	23.5	17.5
10	33.5	29.2	23.3	18.5
11	33.0	28.9	24.0	18.7
12	34.7	28.2	23.5	18.5
13	34.7	28.9	23.3	18.3
14	32.4	28.7	24.2	18.4
15	32.5	30.3	23.6	18.6
16	33.9	30.2	24.1	17.8

\* Moisture contents in per cent—dry basis.

Although the point at which the maximum moisture content occurred is not the same distance above the water table in the four columns of the same size, there is considerable evidence that when columns with cross-sectional areas

of from 1 square inch to 16 square inches are considered, the zone of maximum moisture content becomes higher as the columns become larger. Increasing the size of the container to areas greater than 16 square inches seems ineffective in creating a further rise in the zone of maximum moisture content.

#### OBSERVATION UPON UNIFORMITY OF MOISTURE CONTENT WITHIN THE SAME HORIZONTAL PLANE

Column 123 was intensively sampled to determine whether a uniform rate of rise has been experienced at all points within the same cross section in the column. It may be assumed that if the greater rise exhibited in the larger columns were due to the greater rate of rise in the thin layer of soil lying in immediate contact with the sides of the column, a higher moisture content would be found in samples taken from this layer than in samples coming from the center of the column but in the same horizontal plane. Conversely, retardance toward rise caused by the material of the container would be reflected in a lesser moisture content near the sides of the container than in the center of the column.

In table 6 the results of soil moisture samples from planes at varying distances from the water table are given. When the sampling reported previously had been continued from the top of the column to the point 4 feet above the water table, the soil remaining above this point was removed. The top of the column remaining was divided into 16 equal parts by lines drawn with a spatula. A sample of the soil from the center of each of these 16 subdivisions was then obtained by boring downward a distance of 3 inches with the cheese sampler. These samples then represented the moisture content in the column under consideration, for the zone which extended from 45 to 48 inches above the water table. The locations within the plane are designated as follows:

Back of column

4 - 3 - 2 - 1

5 - 6 - 7 - 8

12 - 11 - 10 - 9

13 - 14 - 15 - 16

---

Glass face of column

The moisture content was determined as previously described and is expressed as percent on the oven-dry basis.

The same procedure was followed in planes 3 feet, 2 feet, and 1 foot above the water table. The results of these observations are given in table 6. The locations refer to the key given above.

A study of the moisture contents at various points found in these four planes



of column 123, does not indicate any significant differences of moisture content within the same plane. The greatest differences found in the 16 locations in the same plane were in the plane 1 foot above the water level. Samples for this determination were taken from a zone from 9 to 12 inches above the water level. In this plane the highest moisture contents were not found to be segregated in the center of the plane nor along the sides of the column. Reference to table 5 indicates that the maximum water content in column 123 was found at a point 20.7 per cent of the total distance from the water table to the elevation of maximum rise. Since the maximum rise in column 123 was almost 5 feet, it is evident that samples taken from a plane 1 foot above the water level come from the zone of maximum moisture content. This understanding may explain the erratic nature of the results from the 1-foot plane. It seems fair to conclude from the data presented that the moisture was fairly uniformly distributed within the various planes and that there was no consistent difference in moisture content between the samples taken from the center of the columns and those taken near the sides of the columns. There is some evidence also that the heights of rise as noted through the glassed fronts of the columns are a fair index of the moisture movement upward by capillarity.

No satisfactory explanation of the causes for the results listed above has occurred to the authors at this time. The evident suggestion that the cause is variation in temperatures on different parts of the column does not completely satisfy all the conditions, since in the preliminary observations when all the columns were maintained in a constant temperature chamber there were indications that the size of the container was significant in determining the capillary rise of water through soil columns. In the later installation every effort was made to eliminate wide variations in temperature between size groups in the bank of soil columns. Convenience of installation made it desirable that columns of the same size be scattered throughout the assembly instead of placing all the individuals of a size together. It seems doubtful if localized differences in temperature could effect such a consistent variation as is reported in rise among the sizes.

The suggestion that the lesser rise in the smaller columns is due to greater frictional resistance by the sides of the columns is perhaps logical. Careful scrutiny will show, however, that the degree of retardance in the smaller columns has no relation to the ratios of cross-sectional areas of columns to their perimeters. This suggestion is still further disputed by the uniformity of moisture content in the cross section of a column. Preliminary observations with cylindrical columns of celluloid showed results of the same nature as those later reported. It is probable that the material in which the soil columns are contained is of minor importance, and if so it is probable that the resistance to rise because of friction on the container may be disregarded.

Colloidal changes within the soil mass, due to the progressive wetting, are probably the controlling influences. Still the evidence given by Bouyoucos (1) shows that when soils are unequally moistened the swelling of certain types

of soil colloids tends to set up unequal stresses. In the case of the smaller columns these stresses would probably result in greater compactness than in the larger columns because of the greater perimeter per unit area of cross section. Increasing the compactness in a column would supposedly reduce the effective diameters of the capillary channels and result in a greater ultimate rise, but would reduce the rate. Such, however, was not found to be the case in the columns under consideration. The smaller columns not only rose more slowly than the larger columns but reached a maximum rise after one-third of the period of observation had elapsed.

It is hoped that this problem can be still further investigated in the near future.

#### SUMMARY

1. Evidence is furnished that the extent of capillary rise through soil masses from a free water table is affected by the cross-sectional area of the column under consideration.

2. In general large columns show a greater rise after a given time than small columns.

3. From the observations reported, size of the container is of greatest importance in columns with a cross-sectional area of less than twenty-five square inches.

4. Intensive soil moisture samples indicate that there is no uniform distribution of moisture throughout the length of the capillary columns. A point or zone of maximum moisture content is found at an appreciable distance above the water table.

5. There is some evidence that in columns of small cross-sectional areas the distance of this zone of maximum moisture content above the water level varies with the size of the column, this distance being greater as the columns become larger. When the cross-sectional areas of columns become greater than about sixteen square inches, further increases in size do not affect the relative position of this zone of maximum moisture content.

6. Moisture samples taken at various points in the same horizontal plane within the column indicate a fairly uniform and consistent moisture content at all points.

7. No experimental evidence is available to support the belief that the upward rise as indicated through the glassed face of a capillary column is not indicative of the rise within the whole soil mass.

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#### PLATE 1

FIG. 1. Detail of procedure used in filling capillary rise columns.

FIG. 2. Section of capillary column assembly used to demonstrate the effect of the size of the container upon capillary rise from a free water table. The photograph shows the extent of rise after 16 days.

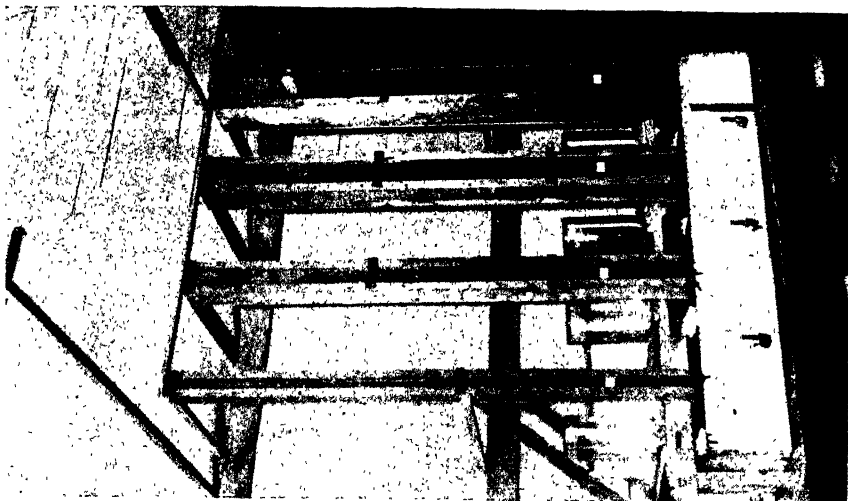


FIG. 2



FIG. 1



## REVIEW OF GERMAN LITERATURE ON SOIL SCIENCE AND PLANT PHYSIOLOGY IN 1925<sup>1</sup>

Received for publication February 18, 1926

### SOIL SCIENCE

BALKS, R. *Investigations on the formation and decomposition of humus in the soil.* (Landw. Vers. Sta. 53: 221-258.)

The author investigated samples from six different soils which had been heated with stable manure. The formation and decomposition of humus in the soil were determined quantitatively at different times.

BLANCK, E., AND ALTEN, F. *Contributions to characterization and classification of "Roserde."* (Landw. Vers. Sta. 53: 41-72.)

The authors described the way in which "Roserde" had been classified up to the present. The following conclusion was drawn from their own investigation:

When for a sample of "Roserde" the hygroscopicity and the amounts of  $Al_2O_3$  and  $Fe_2O_3$  soluble in HCl were determined it was possible to state whether the soil should be classified as belonging to the Mediterranean or to the tropical type of "Roserde."

BLANCK, E., AND ALTEN, F. *Experimental contributions on the formation of "Roserde."* (Landw. Vers. Sta. 53: 73-90.)

The authors carried out leaching experiments and came to the following conclusion:

Magnesite is of little importance for the formation of "Roserde." Precipitation of iron may be prevented by the presence of colloidal humus substances even if lime is present at the same time.

BOBKO, E. W., AND DRUSCHININ, D. W. *Influence of certain factors upon the reaction of soil solution.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 5: 45-369.)

The authors investigated the influence of different soil water ratios upon the soil solution. They compared the pH values of water extracts with those of soil solutions. Further, they discussed the influence of lime application upon soil reaction.

FRESENIUS, L. *The present state of the question of soil acidity.* (Ztschr. Pflanzenernähr. u. Düngung. (B) 4: 200-212.)

<sup>1</sup>These abstracts were prepared by Chr. Krull and E. A. Mitscherlich, University of Königsberg, Prussia, and translated from the German by S. T. Jensen, New Jersey Agricultural Experiment Stations.

The author reviews the different questions in the problems of soil acidity and the corresponding methods for its determination.

GEHRING, A., AND WEHRMANN, O. *Studies on the effect of lime upon soils.* (Landw. Vers. Sta. 53: 179-335.)

The authors studied in the laboratory the effect of "Endlauigekalk" and "Kalikalk" (industrial waste products) upon the physical and biological conditions of the soil. Further they studied in the field the effect of various kinds of lime upon the crop yield and upon the  $\text{CO}_2$  production of soils. Finally they determined the degree of saturation of different soils with lime according to Hissink's method. They reported their own method for determining the maximum combining power of a soil with lime.

GEHRING, A., AND SCHÜLKE, G. *On the effect on soils of some natural varieties of lime and marl and of some Ca and Mg compounds.* (Ztschr. Pflanzenernähr. u. Düngung. (B) 4: 113-139.)

The authors investigated the solubility of different varieties of lime of different degrees of coarseness. Further, the influence of  $\text{CaO}$ ,  $\text{CaCO}_3$ ,  $\text{CaSO}_4$ ,  $\text{MgO}$ ,  $\text{MgCO}_3$ , and  $\text{MgSO}_4$  upon the physical, chemical, and biological processes in soils was studied.

HAGER, G. *On the determination of acidity in mineral soils.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 159-177.)

The author discussed the different forms of acidity. He concluded from his experiments, that Kappen's explanation of the exchange acidity is correct.

HISSINK, D. J. *The saturation condition of the soil: A. Mineral soils (clay soils.)* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 137-158.)

The author studied first the saturation condition of a number of clay soils as far as exchangeable bases were concerned, and then the question of liming.

HISSINK, D. J. *The method of mechanical soil analysis.* (Internatl. Soc. Soil Sci. Proc. 1:137-156.)

The author first discussed the preliminary treatment of soil samples with  $\text{HCl}$ ,  $\text{H}_2\text{O}_2$ , and  $\text{NH}_4\text{OH}$ , before the sedimentation process in an Atterberg cylinder was carried out. Then he recommended a new method for preliminary treatment as worked out in his laboratory. Finally he discussed the sedimentation methods devised by Wiegner, Odeir, Robinson and Kraus.

KAPPEN, H., AND BELING, W. *On the quinhydrone method, the relation between its results and the different kinds of soil acidity.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 6: 1-26.)

The authors tested Büllmann's quinhydrone method for electrometric pH determinations. Then by means of this method they studied the exchange

acidity and the hydrolytic acidity of a large number of soils. A number of very interesting results, from the point of view of the method, were obtained.

KAPPEN, H., AND BOLLENBECK, K. *On the importance of the different kinds of soil acidity for making little-soluble phosphates more soluble.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 1-29.)

The authors used the following acid substances in their experiments: Humic acid from sugar obtained by Berthelot's and Andre's method, natural humic acid, amorphous  $\text{SiO}_2$ , permutite and field soil possessing exchange acidity. The dissolving effects of the three kinds of acidity upon phosphates were studied in those substances. The phosphates used in the experiments were tricalcium phosphate and natural phosphorites.

KNICKMANN, E. *Investigations on the question of soil acidity.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 5: 1-92.)

Having discussed the present state of the soil acidity problem, the author studied the appearance of the different forms of acidity in profiles from clay, sand, and humus soils. The methods of determinations were critically discussed. The development of the effect of lime upon exchange acidity and hydrolytic acidity was studied experimentally.

KNICKMANN, E., AND HELBIG, M. *Investigations on soil exhaustion.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 5: 209-248).

The authors studied the physical and chemical properties of exhausted forest soil and of poor humus. In this connection investigations on soil acidity were made.

NICKLAS, H., AND HOCK, A. *On the question of exchange acidity in soils and the relation between titration acidity and actual acidity.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 5: 370-392.)

The authors studied the behavior of the exchange acidity for pure solutions of Al salts and for a number of different kinds of soils. The corresponding relations between titration acidity and actual acidity were also studied.

RENNER, W. *The influence of various fertilizers including lime and phosphates upon the structure of the soil.* (Ztschr. Pflanzenernähr. u. Düngung. (B) 4: 417-451).

The author studied the effect of  $\text{CaO}$ ,  $\text{CaCO}_3$ ,  $\text{CaSO}_4$ , superphosphate, Thomas phosphate, and Renania phosphate upon the structure of different soils. He concluded from his experiments that  $\text{CaSO}_4$  and super phosphate seemed to have an unfavorable effect upon soil structure as opposed to that of the other fertilizers.

SCHEFFER, F. *On the nature and causes of the transformation of burned lime in the soil.* (Jour. Landw. 72: 201-235.)



The author summarized his experimental results as follows:

1. In soils with a small lime content the transformation of  $\text{CaO}$  into  $\text{CaCO}_3$ , as far as studied, does not take place quantitatively even with neutral reaction. A large amount of  $\text{CaO}$  is taken up in another way.
2. The experimental results of E. Blanck and G. Hager were completely substantiated.
3. Probably a complete transformation of  $\text{CaO}$  into  $\text{CaCO}_3$  takes place only in soils containing an appreciable amount of  $\text{CaCO}_3$ .
4. Completion of the quantitative transformation is prevented by the presence of absorbing substances in the soil.
5. It was possible to demonstrate that silica gel and the mixture gel  $\text{SiO}_2\text{-Al}_2\text{O}_3$  were absorbing substances.
6.  $\text{SiO}_2$  gel is capable of decomposing  $\text{CaCO}_3$ .

#### PLANT PHYSIOLOGY

ARND, TH. *The humic acids, their influence upon the life of micro-organisms in peat soils and the methods of acidity determination.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 53-72.)

By determining ammonia formation, nitrification, and denitrification, the author studied the influence of humic acids upon the life of microorganisms in different kinds of peat. The peat samples used were moor peat, heath peat, and low peat bog.

ARRHENIUS, O. *The lime requirement of soils from a plant physiological viewpoint: II. Soil reaction and the growth of higher plants.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 30-52.)

The author continued his review on this subject published earlier in *Zeitschrift für Pflanzenernährung und Düngung*. He called attention to the fact that different plant varieties are different in their behavior to soil reaction. The paper contains an extensive review of the literature.

ARRHENIUS, O. *The lime requirement of soil: III. The influence of soil reaction upon biological physico-chemical soil factors.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 348-358.)

Using his own experiments and those of other investigators, the author demonstrated the great importance of liming and of soil reaction for all factors of soils.

ARRHENIUS, O. *The lime requirement of soil: IV. Practical applications of the study of soil reaction.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 5: 195-199.)

The acid and alkaline conditions of soils from certain estates were presented graphically on a colored map. It was thus demonstrated how the study of soil acidity could be used for practical purposes.

DENSCH AND HUNNIUS. *Studies on the growth of oats. The water content of the soil at different times during the period of growth and its influence on crop*

yield. *The ratio between grains and straw and the assimilation of plant nutrients, especially phosphoric acid.* (Landw. Vers. Sta. 53: 91-102.)

Assimilation of phosphoric acid, of potassium, and of nitrogen could be observed, even at the end of the period of growth, when water supply was abundant. In dry periods assimilation, especially that of nitrogen, was much decreased.

KAPPEN, H., AND LUKACS, M. *On the physiological reaction of chemical fertilizers.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 249-270.)

The authors grew cultures of corn, mustard, and buckwheat in sand cultures and in culture solutions. They studied the effect of the different plants upon the reaction of the medium in which they were grown.

KIRSTE, H. *On the growth of plants in acid soils.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 5: 129-194.)

The author studied the conditions of soil acidity and its influence upon plant growth. Extensive field and pot experiments were carried out with different cultivated plants. Various kinds of fertilizers and increasing amounts of lime were applied.

KRULL, CHR. *Remarks on Reinau's investigations on carbonic acid.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 359-367.)

The author discussed and criticized Reiman's work of 1924 on the CO<sub>2</sub> question.

LEMMERMANN, O., AND WIESSMANN, H. *Studies on the increases in crop yield due to silica.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 4: 265-315.)

When plants are grown without a sufficient supply of phosphoric acid, an addition of silica will increase the crops. The authors explain this fact by the dissolving effect of silica upon phosphates.

MEVIUS, W. *The direct influence of H-ion concentration in culture mediums upon plant cells.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 6: 89-98.)

The author studied the hydrogen-ion concentration and its constancy in the cell sap, and the relation between the pH values in the plant cells and in the culture solutions. He concluded from his experiments that the effect of H-ion concentration in a culture solution is controlled by the permeability of the cell.

MITSCHERLICH, E. A. *The strain and variety experiment and its influence on the methods of plant breeding.* (Mitt. Deut. Landw. Gesell. 50.)

The author discussed the methods used hitherto in field experiments with different strains and varieties of plants. He claimed the necessity of following new lines in plant breeding, for instance: I. Breed plants which require

certain amounts of water at definite times during the growth period. II. Breed plants resistant to acid or alkaline soil reaction. The author tested the effect of reaction upon two different strains of oats and barley, and demonstrated that pot experiments are well suited for such tests.

MITSCHERLICH, E. A. *Plant physiological investigations on soil acidity.* (Landw. Vers. Sta. 54: 36-42.)

Fifty soil samples were investigated. From the results the author concluded that no correlation exists between the actual acidity and the buffering power of soils. He emphasized that only by vegetation experiments would it be possible to obtain a clear picture of the reaction conditions in the soil.

MITSCHERLICH, E. A. *A contribution to the question of CO<sub>2</sub> as fertilizer.* (Angew. Bot. 7: 24-40.)

The author argued against Reinau's work (1924), mentioned above, on the CO<sub>2</sub> question. He discusses his own standpoint in regard to that problem and the work of Janert, Spirgatis and Lamberg on light and CO<sub>2</sub> as factors of growth.

MITSCHERLICH, E. A. *On the method of determining fertilizer requirements of the soil: II. By the Mitscherlich method.* (Ztschr. Pflanzenernähr. u. Düngung. (B) 4: 193-199, 473-478.)

In two articles (*Zeitschrift für Pflanzenernährung und Düngung.* (B) 4: 25-31, 273-285.) Gerlach criticized the plant physiological method devised by the author for the determination of plant nutrients in the soil. The author argued against this criticism.

PRIANISCHNIKOV, D. N. *On the physiological character of ammonium nitrate.* (Ztschr. Pflanzenernähr. Düngung. (A) 4: 242-250.)

The author carried out experiments with sand cultures and with nutrient solutions. He concluded from his experiments, that the physiological acidity of ammonium nitrate was more predominant than its amphoteric properties.

REINAU, E. *Carbonic acid from soils and from the atmosphere as factors in agriculture.* (Ztschr. Pflanzenernähr. u. Düngung. (A) 5: 393-395.)

The author argued against Krull's criticism<sup>2</sup> of Reinau's CO<sub>2</sub> theories.

SMITH, W. *Carbonic acid as a stimulant and as building material.* (Ztschr. Pflanzenernähr. u. Düngung. (B) 4: 162-171.)

The author discussed the investigations of Lundegardh and demonstrated by his experiments the effect of CO<sub>2</sub> as a stimulant.

<sup>2</sup> See KRULL, CHR. *Remarks on Reinau's investigations on carbonic acid*, p. 7 of this paper.

USCHIDRAWWEITS, H. *Stimulation experiments*. Dissertation, Königsberg, Prussia. (Bot. Arch. 1925: 119-133.)

The author carried out stimulation experiments for a series of plants. The materials used were  $MgSO_4$  and  $MnSO_4$ . The increase in crop yield reported by Popoff was not obtained.

WAGNER, H. *The relation of plant growth to physical chemistry*. (Landw. Jahrb. 62: 785-808.)

The author carried out vegetation experiments with oats. The plants were grown in pot cultures and the soil was kept at its maximum water-holding capacity. The course of growth was studied when different amounts of water were available. With Mitscherlich's law as a basis, the relation of plant growth to physical chemistry was discussed.

WALTER, H. *The saturation of plants with water, and its importance for plant growth*. (Ztschr. Pflanzenernähr. u. Düngung. (A) 6: 65-88.)

The author discussed investigations on the swelling of the protoplasm of lower plant organisms. He demonstrated that the life processes of higher plants depends in a similar way upon the degree of water saturation of cells. The same, of course, holds true for the crop yield of our cultivated plants.

WEISS, F. *The action factor when the action law of the growth factors is applied to the drill distance of cultivated plants*. Dissertation, Königsberg, Prussia. (Bot. Arch. 1925: 377-385.)

The author applied Mitscherlich's action law of the growth factors to the drill distance between plants. The relation of the action factor to different kinds of cultivated plants was studied.



# ON THE ORIGIN AND NATURE OF SOIL ORGANIC MATTER OR SOIL "HUMUS": II. METHOD OF DETERMINING HUMUS IN THE SOIL<sup>1</sup>

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The methods commonly employed for measuring quantitatively the soil organic matter or soil "humus" are based upon:

1. The determination of total carbon in the soil; the quantity thus obtained is multiplied by 1.74 to give the soil organic matter; 2. the treatment of the soil with an oxidizing agent, such as dilute  $H_2O_2$ , permanganate, or silver chromate; the quantity thus obtained is presumably the more available part of the soil organic matter; 3. the treatment of the soil with a dilute alkali solution; this method presumably allows differentiation between natural organic matter added to the soil and the soil organic matter or "humus."

The last method has come into general use, especially in connection with the study of the nature of the soil organic matter. However, by varying the kind of alkali used for the extraction of the soil organic matter, or the concentration of the alkali, and the length of its action upon the soil as well as the preliminary treatment of the soil with other reagents, investigators obtained varying results. As a result of this, the determination of total carbon in the soil, either by dry combustion or by wet combustion (by means of chromic and sulfuric acids), is frequently used for measuring the soil organic matter. This method is suitable for measuring the total organic matter of the soil, but in a study of the origin and nature of this organic matter, a method had to be used whereby one could differentiate between different constituents of organic materials and especially between the natural organic matter and the soil organic matter. The use of alkalies for the extraction of the latter also offered a means of separating it into several fractions. Whenever methods were available for determining quantitatively definite chemical elements of compounds, they have been utilized.

The soil organic matter, whether from mineral or peat soils has been commonly divided, on the basis of solubility in alkali solutions, into several fractions. The relative amounts of the different fractions depend largely upon the nature of the alkali and its concentration. Since different reagents have been used by different investigators, there is no basis whatsoever for comparison between the results thus obtained. When sodium hydroxide is used for

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extracting the soil "humus" and the solution thus obtained is neutralized with hydrochloric acid, a precipitate is formed. When an excess of acid is added over that necessary for the neutralization of the alkali, a part of the precipitate goes again into solution. When the precipitate is washed with sufficient acid, an organic preparation practically free from inorganic matter is obtained. This preparation has been usually referred to as "humic acid" and has frequently been further subdivided, on the basis of solubility in alcohol or pyridine. However, since no definite proof has been submitted that these substances are definite chemical complexes and as the available information seems to point to the fact that they consist of more than one constituent, it may be convenient to term the part of the soil organic matter soluble in alkalis and precipitated by acids as the  $\alpha$ -fraction of the "soil organic matter." When the acid solution, obtained after the  $\alpha$ -fraction has been removed, is neutralized with an alkali, another precipitate is formed which is soluble both in alkalis and in acids and which can be termed conveniently the  $\beta$ -fraction of the soil organic matter. This fraction is usually absent in peat soils and is found abundantly in mineral soils; it contains about 30 per cent organic and about 70 per cent inorganic materials, largely aluminum, as shown by the carbon, nitrogen, and ash content.

The use of the colorimeter for comparing the alkaline extract of soils may perhaps be satisfactory for measuring the "humic acid" content of peat soils, where the  $\alpha$ -fraction is predominant, but it cannot be applied to ordinary mineral soils, which contain different proportions of the  $\alpha$ - and  $\beta$ -fractions, the latter being only light brown in color and not black as the  $\alpha$ -fraction is.

Soil organic matter can be thus divided into 4 distinct groups of substances, some of which possess well defined chemical characteristics:

1. Those substances which are insoluble in dilute alkaline solutions even after prolonged extraction, either in the cold or at 100°C. Here belong various constituents of natural organic matter, which have not yet been decomposed or which are still in the process of decomposition, and certain substances of microbial origin, such as chitin and various mycohexosans. This fraction of soil organic matter contains the so-called "humus coal" or substances of a high carbon and a low oxygen and nitrogen content and has been referred to in the older literature as "humins" or "ulmins."

2. That part of the soil organic matter which is soluble in dilute alkali solutions (1.5 to 5.0 per cent NaOH), after a shorter or longer period of extraction in the cold or under pressure, and which is precipitated by an excess of hydrochloric acid. This group comprises the so-called "humic acids" ( $\alpha$ -fraction) and is characterized by a definite nitrogen content, usually ranging from 2.0 to 4.0 per cent, with an average of 3.0 to 3.5 per cent, and by a low ash content (about 1 per cent); certain "humic acids" from fresh peat and fresh forest mold may contain a much lower amount of nitrogen for the same amount of carbon. This group received the attention of the great majority of investigators of soil organic matter, as has been pointed out. Some claimed (1) that it is a colloidal substance very complex in composition; others (3,6) tried to prove that it is a pure chemical substance of a comparatively simple structure and of a low molecular weight. The recent theories seem to indicate that it may be largely lignin in nature, although its nitrogen content has not yet been accounted for.

3. Those substances which are soluble in alkalis (1.5 to 5.0 per cent NaOH) and in hydrochloric acid, but are precipitated at a definite isoelectric zone (pH 4.6 to 5.0). This is

an organic-inorganic complex ( $\beta$ -fraction) and is characteristic of mineral soils. Its presence was recorded in the soil by some investigators (7, 4) but no reference is found in the literature concerning its abundance in the soil, its relation to the  $\alpha$ -fraction ("humic acids"), or its rôle in the soil. The ash content of this preparation consists almost entirely of aluminum. The total carbon content of the preparation is about 15 per cent and the total nitrogen 1 per cent, giving a carbon:nitrogen ratio similar to that of the  $\alpha$ -fraction. The substance is probably an aluminum-organic compound ("Al-humate"). It is of interest to note here that by the use of ammonium hydroxide, no  $\beta$ -fraction is obtained, although Blanck and Alten (2) have shown that the treatment of soil with  $\text{NH}_4\text{OH}$  results in considerable losses, largely  $\text{Al}_2\text{O}_3$ . The nature of the  $\alpha$  and  $\beta$  fractions of the soil organic matter will be discussed in detail later.

4. Those substances which are made water-soluble as a result of the alkali treatment—the so-called "crenic" and "apocrenic" acids or "fulvic" acid.

The last three groups of substances comprise the so-called soil "humus" and may amount, if the extraction with alkali is continued long enough, to 60 to 85 per cent of the total soil organic matter, as measured both by the carbon and nitrogen content. The terms soil "humus" and alkaline extract of soil have become almost synonymous, although some investigators still speak of "humus" as the total soil organic matter. The process of "humification" is usually spoken of as the transformation of natural organic matter into the more resistant soil organic matter or the organic matter soluble in alkalies.

The fact that natural materials contain only 15 to 30 per cent of substances soluble in alkalies and not soluble in water (except some hemicelluloses), while the soil organic matter contains 60 to 80 per cent of such materials, would seem to point to a distinct difference in the nature of the substances and to certain specific processes which bring about the change from the natural organic matter into the soil organic matter.

The solubility of a large part of the soil organic matter in alkalies offers a good starting point for the investigation of its nature. Even if not all the soil organic matter is soluble and even if different soils vary in the amount that becomes soluble, it is still the best, if not the only, general method which differentiates definitely between the larger part of the plant residues, for example, and the so-called soil "humus."

After it has been decided, however, to use the solubility of the soil organic matter in alkalies as an index of the "humus" content in the soil, or of the amount of dark colored organic matter which has become an integral part of the soil and which does not decompose at all or with great difficulty, it still remains to determine how to use this method so as to obtain always comparable quantitative results. A survey of the literature, presented previously, shows that there is a total lack of agreement as to the comparative value of the different methods for the determination of "humus" in the soil. Many students of soils, including soil chemists, seem to have become so much discouraged with the unsatisfactory results obtained from these determinations that they have given up altogether the determination of "humus" in the soil and have limited



themselves to the determination of total carbon. Witness the fact that, after prolonged discussions on the subject, the method of "humus" determination has been excluded entirely from the more recent editions of the "Official Methods of the Agricultural Chemists."

This lack of agreement concerning the method of determination of a substance or a group of substances which lie at the very basis of soil fertility and soil science in general, is due largely to the lack of sufficient knowledge as to just what is being determined. As a result, there was no criterion for determining the efficiency of the various methods. The lack of comparable methods served further to becloud our conception of the nature of the soil organic matter. Certainly the various names and formulae did not serve to advance our understanding of this subject.

The method to be decided upon in the following pages would, of course, be only arbitrary, until some more definite information is obtained concerning the origin and nature of the soil "humus." In the following studies on the origin of this substance or group of substances in the soil, however, as some method had to be employed for measuring quantitatively the formation, accumulation, and decomposition of this "humus" in the soil, the method which would allow the determination of the largest amount of this material, even if only an arbitrary part of it, had to be selected.

#### EXPERIMENTAL

The following studies were outlined with the idea of determining the nature of the alkali to be used for extracting the soil "humus;" its concentration, and the length and nature of its action which would give the largest yield of "humus" from different soils. Of the various ingredient elements of this organic matter, nitrogen and carbon were most important in this study. The ash content and its nature were left out of consideration for the present.

"Humus" is commonly believed to exist in the soil in a form combined with bases, as humate. Preliminary treatment of the soil with hydrochloric acid is supposed to break up the humates, liberating the free "humic acid," which is then extracted by the alkali treatment. The first three experiments reported here deal with the influence of the preliminary treatment of soil with 1 or 2 per cent HCl upon the amount of "humus" extracted by alkalies. The acid treatment was continued for 2 hours, followed by washing with distilled water. Either 5 per cent NaOH or 4 per cent  $\text{NH}_4\text{OH}$  was then added (100-cc. portions to 100 gm. of soil) and allowed to act for 48 hours in the cold. The liquid was then filtered off and, in the case of the NaOH-treated soils, the whole liquid was precipitated with 10 per cent HCl; whereas in the case of the  $\text{NH}_4\text{OH}$ -treated soils, an aliquot portion of the liquid was evaporated to dryness on a water bath, weighed, ignited, and weighed again and the difference taken to represent the amount of "humus" in the soil. The results presented in tables 1 and 2 indicate quite definitely that the preliminary treatment of the soil with 1 or 2 per cent HCl not only does not increase the amount of "humus"

extracted, but tends to decrease it, especially in the case of certain soils and with the 2 per cent acid. The reduction is marked both in the  $\alpha$  and  $\beta$ -fractions. The reason for this reduction can be readily understood when one considers the existence of the  $\beta$ -fraction in the soil, an organic-inorganic complex soluble both in NaOH and in HCl. The preliminary treatment of the soil with acid tends to remove this fraction from the soil and, therefore, also a part of the soil organic matter. In the case of forest and peat soils, preliminary treatment with HCl did not influence materially the amount of "humus"

TABLE 1

*Influence of preliminary treatment of soil with HCl (1 per cent) upon the amount of "humus" extracted from a heavily manured (5A) and unmanured (7A) soil*  
100 gm. of soil extracted with 5 per cent NaOH

SOIL	EXTRACTION WITH HCl	$\alpha$ -FRACTION		$\beta$ -FRACTION		NITROGEN IN SOLUTION AFTER $\beta$ -FRACTION HAS BEEN REMOVED
		Yield	N content	Yield	N content	
		mgm.	per cent	mgm.	per cent	mgm.
5A	+	667		1,236	1.03	28.4
5A	-	805	2.97	1,371	1.35	27.8
7A	+	333		1,226	1.05	9.8
7A	-	353	2.55	1,493	0.87	14.4

TABLE 2

*Influence of ether and acid (2 per cent HCl) upon the amount of "humus" extracted by two portions of 2.5 per cent NaOH from a Sassafras soil*

EXTRACTION	$\alpha$ -FRACTION		$\beta$ -FRACTION		NITROGEN CONTENT OF SOLUTION FROM $\beta$	HCl EXTRACT PRECIPITATED WITH NaOH YIELD
	Yield	N content	Yield	N content		
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
None.....	851	23.6	926	14.8	14.0	
Ether.....	904	25.5	953	16.4	11.7	
HCl.....	406	15.1	572	17.5	7.8	1,716
Ether + HCl.....	423	15.3	584	19.6	9.3	1,847

extracted by alkalis, because these soils contain none or only traces of the  $\beta$ -fraction.

A comparison of NaOH and  $\text{NH}_4\text{OH}$  (table 3) as reagents for the extraction of soil "humus" shows that  $\text{NH}_4\text{OH}$  extracts a smaller amount of "humus" than NaOH; the  $\alpha$ -fraction alone extracted by the NaOH is almost equivalent to the total amount extracted by the  $\text{NH}_4\text{OH}$ . The  $\beta$ -fraction consists of about 30 per cent of organic matter and is extracted only by the NaOH. Filtration of the  $\text{NH}_4\text{OH}$  extract is much more difficult than that of the NaOH extract. The presence of nitrogen in the reagent prevents an accurate analysis of the

nitrogen content of the extracted organic matter. This makes preferable the use of NaOH for the extraction of soil "humus."

As to the influence of concentration of alkali upon the amount of "humus" extracted, the results in table 4 indicate that the use of 2.5 per cent NaOH (100 cc. of reagent for 100 gm. of soil), extracting at 15 pounds pressure for 30 minutes, filtering through paper, then extracting the residual soil with a fresh quantity of 100 cc. of 2.5 per cent alkali, filtering and washing, give the largest amount of organic matter extracted. A 5 per cent alkali solution extracts, under the same conditions, a somewhat greater amount of the  $\alpha$ -fraction, but

TABLE 3

*Amount of "humus" extracted from 100 gm. of soil with 5 per cent NaOH and 4 per cent  $\text{NH}_4\text{OH}$  from a Sassafras soil (A) and a clay soil (B)*

SOIL	ALKALI USED	$\alpha$ -FRACTION	$\beta$ -FRACTION
	mgm.	mgm.	mgm.
A	NaOH	565	1,473
A	$\text{NH}_4\text{OH}$	585	
B	NaOH	588	1,821
B	$\text{NH}_4\text{OH}$	570	

TABLE 4

*Influence of alkali concentration (NaOH) upon the amount of "humus" extracted from a Sassafras sandy soil (100 gm. of dry soil used)*

ALKALI CONCENTRATION	PERIOD OF EXTRACTION	$\alpha$ -FRACTION		$\beta$ -FRACTION	
		Dry weight	Nitrogen content	Dry weight	Nitrogen content
<i>per cent</i>		mgm.	mgm.	mgm.	mgm.
2.5	30 minutes, pressure	910	25.3	1,507	21.2
5.0	30 minutes, pressure	1,060	20.6	1,560	22.7
10.0	30 minutes, pressure	832	13.6	630	4.3
2.5 + 2.5	60 minutes, pressure	895	23.1	2,688	33.8
5.0 + 5.0	60 minutes, pressure	1,038	18.8	1,895	23.4
5.0	48 hours, cold	987	....	1,475	....

of a lower nitrogen content. The greatest amount of nitrogen (56.9 mgm. from 100 gm. of soil) was extracted by the 2.5 per cent alkali repeated twice; a lesser amount was extracted by the single treatment with 2.5 per cent NaOH, and a still lesser amount by the single extraction with 5 per cent NaOH. A 10 per cent alkali solution extracted the smallest amount of the  $\alpha$ -fraction with the lowest nitrogen content and only an inconsiderable amount of the  $\beta$ -fraction. This is due to the fact that the high concentration of sodium tends to coagulate the soil organic matter. A 5 per cent NaOH solution extracted in the cold a somewhat smaller amount of  $\alpha$  and  $\beta$ -fractions than the same solute at a higher temperature.

To be able to determine the balance of the nitrogen in the soil and in the various fractions of "humus" obtained from the soil by treatment with 5 per cent NaOH, the same two soils that were used in the preliminary experiment were selected. The two soils were two plots of the soil fertility experimental series, treated in a like manner for the last 18 years. Every year 5A receives an equivalent of 16 tons of manure per acre, in addition to acid phosphate and potassium chloride, while 7A has received no fertilization during 18 years. Further information on these soils is given by Lipman and Blair (5). (Table 5.)

About 83 per cent of the nitrogen of the original soil is accounted for in the four fractions into which the soil organic matter is divided by the treatment with alkali:

1. The  $\alpha$ -fraction contains about 25 per cent of the recovered nitrogen in soil 5A and about 19 per cent in soil 7A. 2. The  $\beta$  fraction contains about 20 per cent of the recovered nitrogen in 5A and about 33 per cent in 7A. 3. The solution from the  $\beta$ -fraction contains about 30 per cent of the nitrogen in 5A and 29 per cent in 7A. 4. The nitrogen left unextracted in the soil comprises about 24 per cent in soil 5A and about 19 per cent in 7A. Treatment of the soil with 5 per cent NaOH thus extracts 76 per cent of the nitrogen of a well manured soil and 81 per cent of an unmanured soil.

TABLE 5

*"Humus" and nitrogen extracted with 5 per cent NaOH from a manured soil (5A) and a non-manured but cultivated soil (7A)*

(On the basis of 100 gm. of dry soil)

SOIL	CONTENT OF ORGANIC MATTER, BY IGNITION	TOTAL NITROGEN IN ORIGINAL SOIL	$\alpha$ -FRACTION		$\beta$ -FRACTION		NITROGEN IN SOLUTION FROM $\beta$	NITROGEN CONTENT OF RESIDUAL SOIL	TOTAL NITROGEN RECOVERED
			Yield	Nitrogen content	Yield	Nitrogen content			
	<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
5A	4.80	134	825	28.16	1,717	23.01	34.24	27	112.41
7A	2.78	74	318	11.66	1,840	20.33	18.36	12	62.35

The most interesting point in these results is the fact which tends to indicate the difference in the nature of the organic matter in the two soils. The ratios of the nitrogen and the organic matter content of the unmanured to the manured soils are 55.4:100 and 57.9:100 respectively; in other words, the organic matter in both soils, although different in quantity, has proportionally the same amount of nitrogen. On examining, however, the four fractions, in which the nitrogen and the organic matter are distributed, we find that the content of the  $\alpha$  fraction and the water-soluble fraction is distinctly different in the two soils, whereas the amount of the  $\beta$ -fraction is practically the same, both in the amount of organic matter and in the nitrogen content.

These studies were repeated using a larger series of plots, under various treatments. (Table 6.) The results confirmed the observations made previously that differently treated soils belonging to the same soil type have about the same amount of organic matter and nitrogen in the  $\beta$ -fraction. The

differences are found largely in the  $\alpha$ -fraction and in the amount of organic matter remaining in solution and not precipitated by the acid treatment. A comparison of the results obtained from those plots which receive stable manure year after year (5A, 18A and 5B) and from the two corresponding plots

TABLE 6  
*"Humus" and nitrogen extracted with 5 per cent NaOH from a series of soils, under different manurial treatment\**

(On the basis of 100 gm. of dry soil)

SOIL	TREATMENT	NITRO- GEN CON- TENT OF SOIL	$\alpha$ -FRACTION		$\beta$ -FRACTION			NITRO- GEN IN SOLUTION FROM $\beta$
			Yield	Nitro- gen content	Yield	Organ- ic matter	Nitro- gen content	
		<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
5A	Manure	0.134	766	26.0	1,894	598	22.9	36.1
7A	Nothing	0.074	248	8.2	1,468	478	16.2	19.4
9A	NaNO <sub>3</sub> and minerals	0.097	438	15.6	1,688	526	17.5	31.8
11A	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.095	434	14.2	1,833	585	18.2	33.4
18A	Manure, nitrate and minerals	0.132	792	21.8	1,831	579	19.0	37.8
5B	Lime and manure	0.128	674	23.0	1,702	556	19.8	41.8
7B	Lime alone	0.080	293	9.9	1,863	554	14.6	31.6
11B	Lime, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> and minerals	0.078	456	14.0	1,785	539	18.0	37.8
19B	Lime and minerals	0.082	281	9.6	1,812	548	15.1	24.4

\* See Lipman and Blair (5).

TABLE 7  
*"Humus" and nitrogen extracted from peat and muck soils with 5 per cent NaOH*  
 (10 gm. of dry soil extracted twice with 50 cc. NaOH)

SOIL	$\alpha$ -FRACTION IN 10 GM. OF SOIL		NITROGEN LEFT IN SOLUTION
	Yield	Nitrogen content	
	<i>gm.</i>	<i>per cent</i>	<i>mgm.</i>
Humiphous (English peat).....	2.004	2.56	37.2
Peat No. 1754.....	2.131	3.12	67.4
Alphano muck.....	3.483	2.37	31.2
North Jersey muck.....	2.345	3.35	57.0
Florida muck.....	3.150	3.95	68.0
Muck No. 7.....	3.300	2.90	22.0
Forest black humous soil.....	3.980		
New Jersey peat, brown, uncultivated.....	4.060	2.10	70.6
New Jersey muck, black, cultivated, of same origin as preceding peat.....	4.350	1.85	57.8

which do not receive any manure or fertilizer (7A and 7B), shows that the residual effect of the manure results in a decided increase in the  $\alpha$ -fraction of the soil or the "humic acids." Although no general conclusions may be drawn from these meagre results, some suggestion can be made here. The increase in

the  $\alpha$ -fraction, as a result of the addition of organic matter, indicates its origin; the fact that the  $\beta$ -fraction is the same in one soil type, however differently treated, may be due to the fact that this is an adsorption compound and its amount depends on the amount of the absorbing agent, namely the aluminum, which may not vary in the same soil type.

The average nitrogen content of the "humus" or the  $\alpha$ -fraction from an old peat soil was found to be 3.38 per cent. These and results from other experiments tend to indicate that the  $\alpha$ -fraction of ordinary soil is very similar in nature and composition to the "humus" of peat and bog soils. The  $\beta$ -fraction of the various soils reported in table 6 shows a nitrogen content of 3.25 per cent for the organic part of these soils. The organic matter was determined by ignition, then allowance was made for the water lost by the inorganic part of the complex, assuming it to be entirely aluminum hydroxide.

To allow further comparison of the "humus" of ordinary soil and of peat soils, a series of analyses is given in table 7. The "humus" or  $\alpha$ -fraction of peat and muck soils is very considerable, ranging from 20 to 35 per cent; the  $\beta$ -fraction was found to be present only in very small amounts. It is doubtful whether all the "humus" has been extracted from these soils. In some cases as little as 19 per cent of the original material was left in the residue. It is interesting to note that the three preparations having the lowest nitrogen content gave also the lowest amount of nitrogen in solution, after the  $\alpha$ -fractions had been removed.

A black alkali soil (pH 9.8) examined for "humus" gave, per 100 gm. of dry soil, 112 mgm. of the  $\alpha$ -fraction, containing 2.73 per cent nitrogen, and 1225 of the  $\beta$ -fraction, 70 per cent of which was ash, with 61 mgm. of nitrogen in the solution from  $\beta$ . In the nature of the organic matter, the alkali soil approaches normal soils.

To determine whether the nitrogen content of the  $\alpha$  and  $\beta$  fractions is constant or can be changed by treatment with acids and alkalies, 2500 gm. of ordinary sieved field soil was extracted. It yielded 12,145 gm. of the  $\alpha$ -fraction containing 2.84 per cent nitrogen and 1.2 per cent ash, and about 20 gm. of the  $\beta$  fraction, containing 1.08 per cent nitrogen. When 2-gm. portions of the dry material of the two preparations were boiled for 2 hours with 100-cc. portions of 2 per cent HCl, the  $\alpha$ -fraction lost 19.5 per cent of the material, with 12.3 mgm. of nitrogen going into solution; in other words, the part that has become hydrolyzed contained only 3.15 per cent nitrogen or very close to the nitrogen content of the original preparation. The  $\beta$ -fraction lost 30.6 per cent, as a result of boiling with acid, and 7.7 mgm. of nitrogen went into solution, the hydrolyzed portion containing 1.26 per cent nitrogen, or also a trifle more than the original material. In other words, both preparations of soil "humus" are rather stable in composition, the amount of nitrogen being only inappreciably reduced by boiling with acids.

## DISCUSSION

The determination of "humus" in soil was considered at one time to be one of the most important methods in soil analysis, but these determinations are now practically all abandoned. The proceedings of the Association of Official Agricultural Chemists show that where numerous determinations were made ten to fifteen years ago, none are made at present. This is due entirely to the fact that the results obtained by the methods in vogue were altogether too variable to be reliable. It is sufficient to quote Dr. J. H. Petit, who remarked at one of the meetings of the association that

the results go far to confirm the idea that the determination of humus in soils is extremely unsatisfactory . . . . the determination of one definite constituent of soil organic matter as, for instance, carbon is a much better indication of what the soil contains.

The investigations presented in this paper were not undertaken with the idea of reviving the old methods of "humus" determination or of developing new methods. Since the very term "humus" does not stand for any definite chemical complex, one would expect a priori that any method suggested for measuring "humus" would have to be limited to one or more elements, such as carbon and nitrogen. If the term "humus," however, is limited to only that part of the soil organic matter which is resistant to rapid decomposition and if that complex (or only a definite part of it) can be measured by some convenient method, it is essential to investigate this method, before it can be utilized in a study of the origin of "humus" in the soil. As pointed out in the historical review, many of the recent investigators are inclined to consider lignins as at least one of the mother substances of soil "humus." Both of these have very similar properties in their behavior toward reagents. When lignin, prepared from natural organic substances by treatment with concentrated acids, is treated with an alkaline solution, only a part of the lignin, usually not more than 70 per cent, is brought into solution, even under pressure. The greater the concentration of alkali, the more continued the extraction and the higher the pressure, the greater will be the amount of lignin extracted. The same is true of soil "humus." A peat soil treated with gaseous hydrochloric acid was found to contain 70 to 75 per cent "lignin;" the same soil, on alkali extraction, gave only 40 to 45 per cent "humus;" in other words, this peat soil contains an organic complex, comparable to lignin in its chemical behavior and in its solubility in alkalis. In normal mineral soils, the quantity of "humus" is usually 1 to 6 per cent and one has to use the alkali extraction method, if the results thus obtained are comparable.

With these ideas in mind, the method of determination of "humus" in soils was studied. The results obtained are as follows:

1. The method finally adapted for the extraction of "humus" from the soil is fairly reliable, giving comparable results when the same soil is extracted at different times. It is sufficient to cite the results obtained from soil 5A, sampled at four different times of the year.

Three of these samples are reported in tables 1, 5, and 6. These results are 805, 825, 766 and 870 mgm. of the  $\alpha$ -fraction for 100-gm. portions of dry soil, containing 23.91, 28.16, 26.0 and 28.2 mgm. of nitrogen, respectively. The results for the  $\beta$ -fraction vary more because of the high ash content of the latter. This points definitely to the fact that the particular method of measuring "humus" in the soil especially the  $\alpha$ -fraction, or that part of the soil organic matter which is soluble in alkalis and precipitated by an excess of hydrochloric acid, deserves confidence and can be used for future studies. 2. There is great similarity between the  $\alpha$ -fraction in normal soils and the "humus" of peat soils, especially in the case of muck and old peat soils. Fresh peat soils as well as forest "humus" soils contain much less nitrogen, the explanation for which is to be looked for in the origin of "humus" in the soil, as will be elucidated in the following contributions to this subject. 3. The existence of two fractions of "humus" in normal soils, both soluble in alkalis and precipitated by acids, one of which is insoluble in an excess of acid ( $\alpha$ -fraction) and the other of which is redissolved ( $\beta$ -fraction). The first is practically free from ash, containing only 0.8 to 1.5 per cent ash, when properly washed with hydrochloric acid and water; the nitrogen content of the  $\alpha$ -fraction is more or less definite ranging from 2.8 to 3.5 per cent. The  $\beta$ -fraction contains 50 to 70 per cent ash, depending upon the soil and only 0.8 to 1.5 per cent of nitrogen.

#### SUMMARY

1. Preliminary treatment of soil with hydrochloric acid before the extraction of "humus" with alkali is not required for the purpose of obtaining the maximum amount of "humus;" it may actually reduce the amount of organic matter which is extracted by the alkali.

2. Sodium hydroxide is preferable to ammonium hydroxide for the extraction of the "humus" from the soil. The amount of organic matter extracted is greater and the nitrogen content of this organic matter can be determined, without any danger of interference by the reagent.

3. It is proposed to carry out the determination of humus as follows:

The soil consisting of a mixture of several samplings is sieved through a 1-mm. sieve. Six 50-gm. portions of soil are placed in flasks or beakers and 50-cc. portions of 2.5 per cent NaOH solution are added. The flasks are then heated in an autoclave for 30 minutes at 15 pounds pressure or allowed to stand for 48 hours in the cold. The solution is then filtered through folded filter paper. Fresh 50-cc. portions of 2.5 per cent NaOH solution are added to the soil and extraction is repeated. The second extract is filtered through the same paper. The soil is then thrown upon the paper and washed first with 2.5 per cent NaOH solution, then two to three times with distilled water, until the solution comes through quite clear. The combined filtrate is then treated with 10 per cent warm hydrochloric acid, until a heavy precipitate is formed; half as much more acid is then added and the flask is well shaken. A part of the precipitate may go into solution. The precipitate left is filtered through a weighed dry filter paper or a Gooch crucible, washed several times with a 2 per cent solution of HCl, and then several times with distilled water. The precipitate is dried at 65 to 70°C. for 12 to 24 hours and weighed. Three portions of the precipitate are used for the determination of the ash content and three for total nitrogen determinations. The filtrate from the first precipitate is treated with a 2 to 3 per cent solution of NaOH, until a precipitate begins to form; the alkali is then added drop by drop to obtain the maximum precipitation, avoiding carefully an excess of alkali. This second precipitate ( $\beta$ ) is also filtered through weighed dry papers or Gooch crucibles; the filtrate is carefully tested with a drop of acid and of alkali to see that no further precipitate is formed. If it does form, the filtrate is carefully adjusted again and the new precipitate is added to the first. This precipitate is



washed a number of times with distilled water, then dried at 90 to 100°C., and weighed. Two portions are used for ignition, two for total carbon, and two for total nitrogen determinations. The liquid from the  $\beta$  precipitate, which should be straw colored, should be used for the determination of total nitrogen and carbon in solution.

4. In the case of peat soils, only 5-gm. portions of dry soil are taken for analysis and treated with 50 cc. of a 5 per cent NaOH solution twice, as before.

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# THE RELATION OF LIGHT TO SOIL MOISTURE PHENOMENA<sup>1</sup>

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## PART I

### INTRODUCTION

An attempt was made in this laboratory to measure the capillary or pressure potentials of soils by a vapor pressure method. The experiment was carried out by placing small cans of soil in desiccators containing solutions of known vapor pressure. These desiccators were immersed in a constant temperature water bath. It was hoped that by this method the vapor pressures of the soils could be determined and the pressure potentials calculated as will be explained in a later part of this paper. The results were disappointing because the wet soils in desiccators containing distilled water dried out far more than was expected.

After carefully considering all possible causes, the one that seemed to account best for this loss of water was that the soil, being a dark body, absorbed more light and heat radiations than the water, causing the soil to be maintained at a higher temperature. This difference in temperature would cause a distillation of water from the soil to the water in the bottom of the desiccator. Preliminary experiments carried out in this laboratory seemed to indicate that absorption of light was the big factor in the drying noted.

The only other cause that seemed reasonably to account for the drying, was that discussed by Washburn (11). He called attention to a drying of potters clays in damp chambers, and came to the conclusion that, since the clays were at a higher level than the water, the downward distillation was due to gravity. This conclusion was sustained by a preliminary experiment in which he supported wet clay balls in bottles. Some were over water and others were lowered into small open vessels sunk in water so that the clay was below the level of the water surrounding the inner vessel. He reported that the balls above the level of the water dried out, whereas those below the water level absorbed more water.

That this explanation is insufficient is proved by the fact that if a wick or column of soil is placed so as to connect the water and the dried clay ball, the ball will become wet. Water will distill from the wet ball to the free water below, and since the water that is distilled out will be replaced through the wick, there is a motion of water in a cycle. Without differences in temperature in the system this motion would violate the second law of thermodynamics, which states that a system or arrangement of matter operating in a cycle cannot transform heat into work in surroundings of constant temperature. There is, as Washburn concluded, some drying to be expected under isothermal conditions because of difference in height between the

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soil and water, but this is slight. Its magnitude will be discussed in a later part of this paper.

The present paper reports: (a) experiments designed to determine the effect of light on soil moisture phenomena, and (b) a theoretical study of the mechanics of soil moisture, especially in its relation to saturated vapor under varying conditions of light. The theoretical part considers the effect of light on the moisture equilibria between the opaque and transparent portions of the systems considered.

## EXPERIMENTS CONDUCTED

### *Description of absorption apparatus*

To determine just what effect light has on absorption of water vapor by dry soils and distillation from wet ones, a special apparatus was built. It was submerged in a large water bath held at constant temperature by a non-incandescent electric heater controlled by the system described by Thomas (8). By this method the temperature could be held within a range of  $0.002^{\circ}\text{C}.$  as long as the electric power was continuous. The water in the bath was well stirred to insure uniform temperature throughout.

The apparatus (fig. 1) consisted of a 10-inch desiccator with a plate glass top. In the top were drilled one  $\frac{1}{8}$ -inch hole in the center to allow for the stirrer shaft, and six equally spaced  $\frac{1}{4}$ -inch holes on the circumference of a circle of  $3\frac{1}{4}$ -inch radius. In the center hole was sealed a short piece of glass tubing to act as a bearing. Through this was inserted the stirrer shaft carrying a fan for stirring the air, the shaft being bent so as to stir the water. In each of the six holes was sealed a 4-inch piece of glass tubing. Soils to be tested were placed in the lids of standard 3-inch aluminum soil cans suspended on fine copper wires through these tubes. A balance sensitive to 1 mgm. was mounted above the desiccator on a movable horizontal platform, and a fine chain was suspended from the left pan through a hole in the balance case, and was counterbalanced by a weight on the right pan. With this arrangement it was possible to bring the balance over each hole in succession, obtain the direct weight of the soils, and from this calculate their moisture percentages. This could be done at any time without opening or moving the desiccator. On the top of each of the six glass tubes, a short piece of rubber tubing was placed so that except when weighings were being made, each hole was stopped with a glass plug, entirely sealing the desiccator.

The advantage of stirring the air and water, as reported by Wilson (13), is that it requires only about one-tenth to one-twentieth the time to reach equilibrium with stirring as without it, although the stirring does not in any way displace the equilibrium point.

### *Soils used; methods*

Three different washed soils were used for the experiment: Greenville silty clay loam; Trenton clay, a heavy clay soil; and T<sub>1</sub>, a separate of Trenton clay of sizes less than  $0.3\mu$  in radius. These soils have been used for other experiments at this station and their mechanical analyses and vapor pressure-moisture percentage curves are reported by Thomas (9).

The experiment was started using two approximately 5-gm. samples of each kind of soil, one relatively dry and the other approximately saturated. The data are presented almost entirely in the form of moisture percent-time curves, as they are too cumbersome in the tabular form. To determine the accuracy of the data, on several occasions after the weighings were made they were repeated, and in no case did the differences noted amount to a difference of 0.1 per cent in moisture content. All moisture percentages are expressed on the dry-weight basis as determined by heating samples of the soil to  $110^{\circ}\text{C}.$  for 24 hours. Figures 2, 3, and 4 show the results obtained in chronological order. The discontinuities were the result of needs for repair of some parts of the apparatus, so the data of the different figures cannot be connected up.

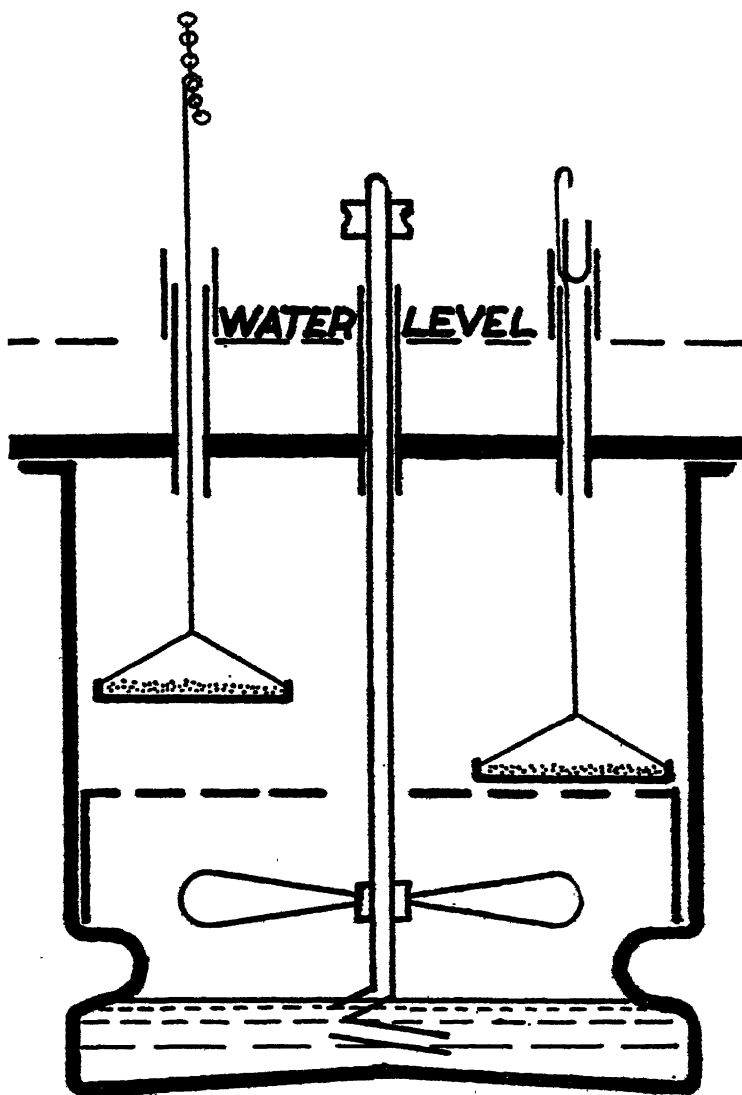


FIG. 1. DIAGRAM OF APPARATUS

The pan on the left is shown in the weighing position, connected to the chain supported from the balance (balance not shown). The pan on the right is in the position in which it is left during the absorption, and the hole in the top is plugged.

#### *Discussion of data*

*Period 1.* In figure 2 are shown the results obtained without absolute darkness. The walls and bottom of the bath were opaque but the top was not entirely covered. The curves became nearly horizontal by the fifteenth

day. At this time the stirrer in the apparatus was stopped and for the remaining 5 days the absorption was practically zero, and the drying was greatly reduced.

*Period 2.* When the apparatus was again started (fig. 3) a test was made for 6 days to see that everything was in good repair. On the fourth, fifth, and tenth days, because of trouble with the electric power, the temperature fluctuated but for the remainder of the period the power was continuous as shown by a recording instrument.

On the sixth day a 200-watt type-C lamp equipped with a reflector was placed about 50 cm. above the apparatus. In one day all the soils except the

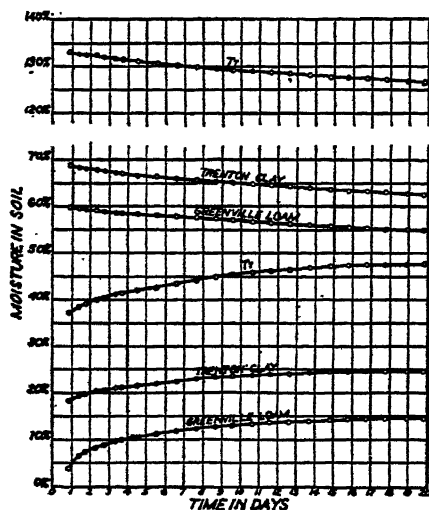


FIG. 2

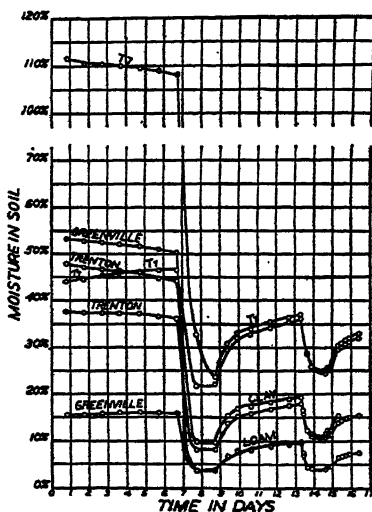


FIG. 3

FIGS. 2 AND 3. MOISTURE TIME CURVES OF THE SOILS IN SATURATED ATMOSPHERE

Figure 2 shows the wetting and drying curves in very subdued light.

Figure 3 shows the effect of strong light in the desiccator, from sixth to eighth and the thirteenth days.

wet T<sub>7</sub> had reached approximate equilibrium, this had lost 3.3 gm. of water reducing its moisture content 75.5 per cent. By the next day all had reached equilibrium. The samples of the clays that had been saturated did not dry as much as those that had been only moist. This was as expected, especially with the heavy soils, as saturating tends to pack the soil closer together and thus allows it to hold a greater amount of water at a given vapor pressure of the soil. This effect was reported by Thomas (9).

On the eighth day the light was turned off and the soils immediately began to absorb water. On the thirteenth day the light was turned on again, left one day, and turned off. Table 1 shows the moisture percentages at selected times during period 2, and compares these with the values of the so-called

hygroscopic coefficient and the wilting coefficient, as determined by standard methods.

These results show that soils will absorb enough water from a saturated atmosphere to bring their moisture contents to values in excess of twice their "hygroscopic coefficients" and to values greatly in excess of their "wilting

TABLE 1

*Per cent of moisture in soils under various conditions of light during period 2, compared with "hygroscopic coefficient" and "wilting coefficient"*

	GREENVILLE LOAM		TRENTON CLAY		$T_2$	
	Wet	Dry	Wet	Dry	Wet	Dry
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
"Hygroscopic Coefficient".....	6.30	6.30	10.2	10.2	*	*
"Wilting Coefficient".....	9.30	9.30	15.0	15.0	*	*
Sixth day, before light was turned on...	50.40	15.80	44.2	36.1	108.2	46.5
Eighth day, light on two days.....	3.74	3.60	9.6	8.1	23.6	22.1
Thirteenth day, light off for five days...	9.90	9.50	19.3	17.9	37.0	36.2
Fourteenth day, light on for one day....	4.40	4.50	12.2	11.2	25.8	25.2
Sixteenth day, light off for two days....	7.50	*	*	15.4	32.9	31.9

\* Data not available.

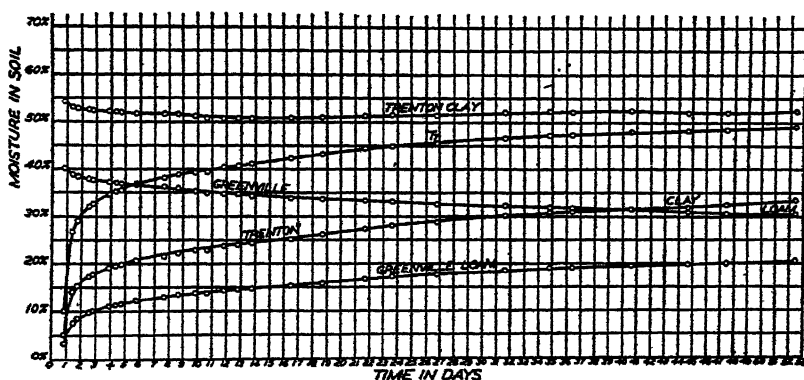


FIG. 4. MOISTURE TIME CURVES OF THE SOILS IN SATURATED ATMOSPHERES

Wetting and drying curves under conditions of less light than in figure 2

coefficients." They show further that in the presence of light a wet soil will dry in a saturated atmosphere to values near or below its "hygroscopic coefficient," and when light is excluded will absorb moisture again.

In this experiment the way in which the moisture content varied with light

intensity leaves little doubt as to the relationship between these two quantities, because as nearly as possible all conditions except light were the same as those during period 1 and the first 6 days of period 2.

*Period 3.* Since from the theory of radiation, all bodies, regardless of color, enclosed in a constant and uniform temperature opaque shell, are at the same temperature, an attempt was made to realize this by enameling the desiccator and covering the bath more completely. The results (fig. 4) were only partially satisfactory because the enamel peeled off opposite the  $T_1$  samples and let considerable light in. That the lower light intensity in the desiccator allowed more rapid absorption of water vapor can be seen from the values of the slopes of the two sets of wetting curves at corresponding moisture percentages. The period taken was from the ninth to the fourteenth days of period 1, and the corresponding range in moisture percentage during period 3. The time chosen from period 1 was the last 5 days before the stirrer was stopped. The slopes are given in table 2.

At the end of period 3 the apparatus was enclosed in a metal case and started again but the power became so irregular that the experiment had to be discontinued.

TABLE 2  
*Slopes of the wetting curves in selected range, taken from figures 2 and 4*  
Expressed in per cent increase per day

CURVES FROM	GREENVILLE LOAM	TRENTON CLAY	$T_1$
Fig. 2, period 1. Desiccator transparent.....	0.29	0.22	0.36
Fig. 4, period 2. Desiccator enamelled.....	0.43	0.45	0.36*

\* Enamel peeled off opposite the  $T_1$  samples, letting light in.

*Calculation of temperature of soil on eighth day of period 2*

By using the data of table 1 and the vapor pressure-moisture percentage curves obtained by Thomas (9), some interesting calculations can be made. To illustrate the method of calculation of the temperature of the soil when the lights were turned on, one set—that for the wet Trenton clay—will be calculated in detail. From table 1 the moisture percentage after the light had been on for two days was 9.6 per cent, which according to the curves just referred to (drying curve) would have at this moisture percentage about 84 per cent of its maximum vapor pressure. The vapor pressure of water at 25°C., the temperature of the bath, is 23.78 mm. of mercury. Since the warm soil was in vapor equilibrium with the water at 25°C., its vapor pressure must have been the same, and from the vapor pressure data this was only 84 per cent of its maximum. So its maximum vapor pressure must have been  $\frac{23.78}{0.84} = 28.30$  mm. This vapor pressure for a saturated soil or for water would be reached at a temperature of 27.95°C. or 2.95°C. warmer than the water in the same

desiccator. Comparison of the other wet soils with their corresponding vapor pressure curves (drying curves) shows that they were at the same per cent of their maximum vapor pressure, and thus at the same temperature. Similar calculation cannot be made for the dry samples, as their previous history was partial saturation and then drying, for which detailed vapor pressure data are unavailable. The curves would be expected to fall between the curves for wetting dry soil and drying wet soil. In every case the range of moisture percentage allowed for 84 per cent vapor pressure between the wetting and drying curves included the value of the corresponding dry sample, on the eighth day.

### *Direct measurement of temperature of soil in light*

As a result of the close checks obtained by the calculations there was little doubt as to their accuracy. To make sure that such differences in temperature could exist in a desiccator immersed in a constant temperature bath, a can of saturated Trenton clay soil was placed in a 5-inch desiccator containing water, and a thermocouple sensitive to  $0.2^{\circ}\text{C}.$  was connected with one juncture in the soil and the other in the water in the bottom of the desiccator. A 200-watt type-C lamp with reflector was mounted 50 cm. above the top of the desiccator, which was 5 cm. below the surface of the water. The temperature of the soil reached a value  $4.5^{\circ}\text{C}.$  warmer than that of the water as compared with  $3.0^{\circ}\text{C}.$  warmer in the previous experiment. This smaller difference in temperature was due to the fact that the tubes in the top of the large desiccator were sealed into the lid with wax, rendering opaque a considerable part of the top.

After the reflector was removed, the soil was found to be only  $0.5^{\circ}\text{C}.$  warmer than the water. A calculation, just the reverse of the one used for the temperature of the soil, based on the wetting curve since the soil had been dried by light, showed that the soil should have had 13.5 per cent water, whereas a direct determination showed 12.9 per cent. The difference is well within the experimental error.

The thermocouple was then replaced by one sensitive to  $0.04^{\circ}\text{C}.$ , and the desiccator exposed to the laboratory light. The experiment was conducted during February in a laboratory with a north exposure. During the day the difference in temperature between the soil and water was from  $0.10^{\circ}$  to  $0.16^{\circ}\text{C}.$  while at night this difference was reduced to practically zero.

### CONCLUSIONS

1. Soils at the moisture content corresponding to the "hygroscopic coefficient," as determined by present methods, will continue to absorb large amounts of water when exposed to a saturated atmosphere in a dark constant temperature chamber. The amount that the soil will absorb is in excess of the moisture content at the "wilting coefficient."

2. The equilibrium point reached under any but isothermal conditions is a function of the light intensity in the apparatus containing the soil.



3. Laboratory light is sufficiently intense to cause serious differences in temperature between transparent and dark bodies.

## PART II. THEORETICAL DISCUSSION

### MECHANICS OF SOIL MOISTURE

The hygroscopic coefficient has been usually considered an equilibrium point: the maximum percentage of water vapor that can be absorbed from a saturated atmosphere. As ordinarily determined the value is about 0.75 of the wilting coefficient.

Bates and Zon (1) and also Robinson (6) reported that it was impossible to obtain equilibrium at relatively low moisture contents. Both accounted for this on the assumption that dissolved substances in the soil solution lower the vapor pressure of the soil below that of free water, and as a result there can be no equilibrium as long as there is any free water at the same level.

In case there are no dissolved substances in the soil solution the problem is different. This condition can never be reached but is approached very closely in washed soils. Various investigators have tried both experimental and theoretical methods to determine under what condition or conditions a soil can be in equilibrium with a saturated water vapor. The results in many cases are contradictory, indicating that in their investigations some essentials have been overlooked.

#### *Saturated soil in equilibrium with saturated vapor*

The deduction of the equilibrium point from the amount of liquid water absorbed by a soil in direct contact with water can be made as follows:

If liquid water and its vapor are in a closed container at constant temperature, the water will distribute itself through the contents according to the laws of hydrostatics. Water will rise in capillary tubes, near solid objects, and also in open spaces between particles of soil. When equilibrium is reached in the liquid phase there must also be vapor equilibrium, for if there were not, water would evaporate at one place and condense at another, thus destroying the liquid equilibrium, and the water would flow back. This would be a motion of water in a cycle, which cannot take place under isothermal conditions. If some of the liquid should become detached, vapor equilibrium would not be reached until all conditions were just the same as if there were liquid communication. If this were not true, after the vapor had come to equilibrium a water connection could be made and there would be a flow of water. This would destroy the vapor equilibrium and there would be a motion of water in a cycle, which is impossible. Presence of air in the system will in no way affect the final equilibrium point but will retard the movement of vapor.

This conclusion was reached by Sir William Thomson (10) who states,

Detached portions of the liquid in separate vessels all enclosed in one containing vessel cannot remain permanently in any other relative positions than those they would occupy, if there were hydrostatic communication of pressure between the portions of the liquids in the several vessels.

It is a well known fact that water will rise in soils and practically saturate them for considerable heights. When equilibrium is reached the soil at any level in addition to being in liquid equilibrium with the water below is also in vapor equilibrium. From this it is safe to say that complete saturation is one condition of the soil for vapor equilibrium between a soil and a saturated atmosphere.

The absorption experiments reported in this paper indicate that there is equilibrium between a saturated vapor and saturated soil. None indicate that there can be equilibrium between relatively dry soil and saturated vapor as some workers have reported. Although the experiments did not indicate a second equilibrium point they did not prove its non-existence, but indicate that if one exists it must be very unstable.

### *Effect of differences in height on equilibrium*

In these absorption experiments the soils were about 10 cm. above the water level in the desiccator. As indicated before, this would tend to make the final equilibrium a little dryer than saturation. Since, when vapor equilibrium is reached, the soil must have the same moisture content it would have if connected with a wick to free water below, the size of pores that can be filled at this height can be calculated. The radius of a tube or pore that can support a column of water of any height  $h$  is given by the formula for rise of water in capillary tubes:  $r = \frac{2T}{hg}$ ; where  $T$  is the surface tension of water, 72.1 dynes per cm. at 25°C.;  $r$  is the radius of the tube or pore; and  $g$  is the acceleration of gravity.

$$r = \frac{2 \times 72.1}{10 \times 980} \quad r = 0.01471 \text{ cm.}$$

Then a pore of 0.1471 mm. radius or 0.2942 mm. diameter would support a column of water 10 cm. high. This means that at this height above a free water surface a soil with no pores larger than 0.2942 mm. would be completely saturated.

### *Derivation of equilibria by geometry and mechanics*

*Previous studies.* Since conclusive proof by experimental methods is difficult, attempts have been made to deduce the equilibrium point by geometry with the aid of mechanics. To do this, it must be assumed that the soil is composed of uniform, insoluble spheres, regularly packed. Wilsdon (12) on these assumptions attempted to calculate the moisture percentage of the spheres when in equilibrium with a saturated vapor. His paper was reviewed, part of the mathematics copied, and a discrepancy pointed out by Keen (4).

Wilsdon started his analysis by considering the water wedge between two equal spheres. He further assumed that the section of the surface of the water

wedge,  $S$  (fig. 5) would be the arc of a circle tangent to the two circles  $A$  and  $B$ . Since the curvature of any surface is the algebraic sum of the curvatures taken on two mutually perpendicular planes normal to the surface at the point in question, the curvature of the water surface will be:

$$c = \frac{1}{r_2} - \frac{1}{r_1} \quad (A)$$

The surface of the wedge must have constant curvature since all parts are in equilibrium hydrostatically. The condition that the wedge be in equilibrium with a saturated vapor is that the surface shall have zero curvature. This condition will be reached when  $r_2$  equals  $r_1$ . By using this condition Wilsdon calculated the central angle  $2\theta$ , subtended between the point of contact of the two spheres and the point of tangency of the wedge, to be  $53^\circ 6'$ . Assuming the closest possible—tetrahedral—packing each sphere would touch twelve

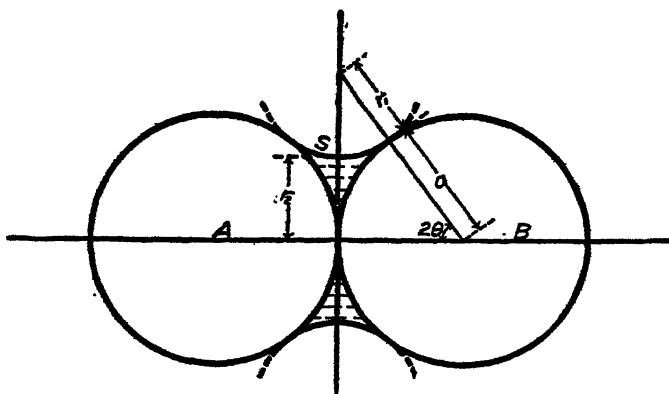


FIG. 5. SECTION THROUGH THE CENTERS OF TWO SPHERES SHOWING WATER WEDGE WITH CIRCULAR SURFACE SECTION

others. By calculating the number of spheres as a function of the radius in 100 gm. of soil, and remembering that there are six water wedges for each sphere in the mass, and knowing the volume of each wedge, he found that the moisture at equilibrium would be 23.95 per cent, which would be independent of the size of the spheres. This value he attempted to correlate with the 21 per cent in the Briggs-Shantz Formulae (2). Keen (4) objected that in any possible regular packing of spheres there would be an overlapping of water wedges before the condition of zero curvature is reached. Thus to an extent Wilsdon's analysis is invalidated. Keen made no attempt to describe the conditions after overlapping.

*Calculation assuming catenoidal surfaces.* The assumption that the surface of the water,  $S$ , is the arc of a circle, leads to a considerable error in calculating the angle  $2\theta$  and the volume of the wedge. The surface of a water film of zero curvature between two spheres can be

shown by mathematics to be a catenoid (a catenary revolved about its directrix). The equation of the catenary with the directrix as the  $X$ -axis and the axis of symmetry, the  $Y$ -axis is:

$$y = C \cosh \frac{x}{C} \quad (B)$$

where by definition

$$\sinh u = \frac{1}{2} (e^u - e^{-u}) \quad (C)$$

and

$$\cosh u = \frac{1}{2} (e^u + e^{-u}) \quad (D)$$

and where  $C$  is the  $Y$ -coordinate of the vertex of the catenary. Before the volume of the wedge can be calculated, the angle of contact between the water and soil must be known. Since for many such substances as glass and quartz the angle of contact is zero or nearly so it will be assumed that the same holds for the soil; that is, that the surfaces of the water and of the soil particle are tangent at the point of contact. With this assumption all that is necessary to evaluate  $C$  of equation (B) is to solve the equations of the catenary and of one of the circles for tangency; that is, equate the values of  $y$  and the values of the slope, or  $\frac{dy}{dx}$ .

The equation of a circle of radius  $a$  tangent to the  $Y$ -axis at the origin is:

$$x^2 - 2ax + y^2 = 0 \quad (E)$$

or

$$y = \sqrt{2ax - x^2} \quad (F)$$

Differentiating (B)

$$\frac{dy}{dx} = \sinh \frac{x}{C} \quad (G)$$

Differentiating (F)

$$\frac{dy}{dx} = \frac{a - x}{\sqrt{2ax - x^2}} \quad (H)$$

Equating the values of  $y$  from (B) and (F)

$$\sqrt{2ax - x^2} = C \cosh \frac{x}{C} \quad (I)$$

Equating the values of  $\frac{dy}{dx}$  from (G) and (H)

$$\frac{a - x}{\sqrt{2ax - x^2}} = \sinh \frac{x}{C} \quad (J)$$

These solved graphically give the following values:

$$C = 0.6815 a$$

$$x = 0.4356 a$$

$$y = 0.8255 a \quad \text{(coordinates of the point of tangency)}$$

$$2\theta = 55^\circ 38'$$

By integration, the volume of water wedge is found to be  $0.463 a^3$ . Assuming, as Wilsdon did, the six independent contacts per particle without overlapping, the moisture would be 27.63 per cent, or 4.19 per cent more than by assuming the arc of a circle; or by assuming the arc of a circle, only 85 per cent of the true value is obtained.

*Relationship with other water wedges.* With cubical packing when the angle  $2\theta$  becomes  $45^\circ$ , and with tetrahedral packing when  $2\theta$  becomes  $30^\circ$ , the water wedges will overlap, so that the analysis breaks down as pointed out by Keen. The question then arises as to the possibility of an equilibrium point after the wedges overlap and before saturation is reached. Assume

that each sphere is surrounded by six others equally spaced as in cubical packing; and that the water wedges could expand, independent of the others, until each comes to zero curvature. At this condition each wedge would have spread  $55^{\circ} 38'$  from each point of contact of the spheres, and since these points of contact are  $90^{\circ}$  apart, wedges on the line between two contacts would overlap  $21^{\circ} 16'$ . This condition would be unstable since adjacent wedges if they had expanded independently would intersect at an angle less than  $180^{\circ}$ , which would smooth out leaving a negative curvature, and not a zero curvature as Wilsdon assumed.

*All spheres covered before zero curvature.* Assuming the loosest possible—cubical—packing there are six contacts on each sphere and these are at the intersections with the surface of the sphere of three mutually perpendicular lines from its center. Take any three of the mutually perpendicular lines from the center to the surface as the positive  $X$ ,  $Y$ , and  $Z$  axes, and a line between, such that its direction cosines,  $l$ ,  $m$ , and  $n$  are positive and equal. The point where this line intersects the surface of the sphere will be equidistant from the points where the axes intersect the surface. The value of  $m$  can be determined, since the sum of the squares of the direction cosines equals unity:

$$l^2 + m^2 + n^2 = 1 \quad (K)$$

$$l = m = n \quad (L)$$

From (K) and (L)

$$3m^2 = 1 \quad (M)$$

$$m^2 = 0.3333 \quad (N)$$

$$\log m = 9.7614 - 10 \quad (O)$$

$$\cos^{-1} m = 54^{\circ} 44' \quad (P)$$

Since for zero curvature the angle  $2\theta = 55^{\circ} 38'$  is greater than  $\cos^{-1} m = 54^{\circ} 44'$ , if the six water wedges should expand independently to zero curvature the entire sphere would be covered and, as previously shown when the angles smoothed out, there would be negative curvatures.

*No equilibrium until saturation.* Between this condition and saturation there will be only air-water surfaces of constant curvature to consider, since all the spheres are covered and it is easily shown that the containing walls would be covered. Since there is air in the soil practically bounded by water, there must be negative curvatures somewhere, and since the curvature must be constant throughout, it must be negative everywhere. There must be negative curvature somewhere since it is obviously impossible to bound a pocket of air with a surface that has everywhere a positive curvature on the air side.

*Application to actual soil.* It is very probable that in practice there would be air trapped in pockets in the soil, but since the air would be bounded by a water surface of negative curvature, it would be under greater pressure than the air outside. Since the solubility of air varies directly with the pressure, the trapped air would slowly dissolve and diffuse out of the soil.

Although the previous discussion holds rigorously only for uniform spheres regularly packed, it should hold fairly well for an actual soil. Soil particles are of variable size and shape but it is reasonable to assume that their contacts are as close as would be the case with spheres packed in the loosest regular condition possible.

Since the experiments reported in Part I of this paper indicate that only when saturated is a soil in equilibrium with saturated vapor, and since a theoretical analysis shows that, at least with uniform spheres under the above conditions, an equilibrium point dryer than saturation is impossible, it seems evident that one does not exist, and that previous workers have failed to reach an isothermal equilibrium with pure water.

*Effect of light on soil absorption and other experiments*

The above analyses do not hold when light is allowed in the apparatus. As was shown in figure 3, when light was turned into the desiccator the soils dried out very markedly. Table 3 based on calculations from the vapor pressure curve of Trenton clay shows the magnitude of the changes in moisture content which will result in a closed vessel from certain differences in temperature between soil and water.

Since in the author's experiments differences in temperature of  $0.16^{\circ}\text{C}$ . between transparent and opaque bodies were noted in winter in a laboratory with a north exposure, it is evident that it is important to conduct absorption experiments in the dark.

Not only are soils experiments affected by light, but also other constant temperature experiments where opaque objects are surrounded by glass and placed in a thermostat. In such cases the difference in temperature between

TABLE 3

*Relation of difference in temperature between soil and water in a closed chamber to the moisture content of Trenton clay*

DIFFERENCE IN TEMPERATURE OF SOIL AND WATER	VAPOR PRESSURE IN PER CENT OF MAXIMUM	MOISTURE IN SOIL
$^{\circ}\text{C}$ .	<i>per cent</i>	<i>per cent</i>
0.87	95	12
0.34	98	17
0.00	100	34-52*

\* Values obtained experimentally. The dry sample absorbed 34 per cent by the end of period 3 (fig. 4), in a saturated atmosphere. As sample was not in absolute darkness and it was still absorbing water when the absorption was stopped, this value would be well below equilibrium value. At the same time under the same conditions, the wet sample contained 52 per cent of water and seemed to be approximately at equilibrium. This value is then probably more nearly correct than 34 per cent.

the opaque and the transparent bodies might be many times greater than the temperature fluctuations allowed in the thermostat. The theory of radiation indicates a means of avoiding trouble from light, since it shows that all bodies, regardless of shape or color, inside of a constant temperature opaque shell, will be at the same temperature.

Since the vapor pressure data of Thomas were used in calculating temperatures of soils in light it might be well to point out that these data were practically free from any light effect. Two methods were used: the dynamic, in which the soils were placed in brass tubes in a thermostat; and the static, in which the soils were placed in desiccators. The latter were placed in well insulated boxes and stored in a dark cellar. The temperature in the cellar would vary not more than  $0.2^{\circ}\text{C}$ . in a week.

*Effect of fluctuations in temperature.* Accurate temperature control in absorption experiments is necessary since dew will be deposited on cooling and

the air will become unsaturated on warming. Since the heating and cooling take place at the outside of the apparatus first, there is a great possibility that the net effect of temperature fluctuations would be a one-way distillation of water displacing the equilibrium point. Many places on the curves (fig. 2, 3, and 4) where points fall below the smooth curve, correspond to a failure of the power or of the control to operate properly. Such places are: figure 3, the fifth, sixth, and tenth days; and figure 4, the eleventh day.

*Effect of light on mechanical stresses in soil moisture.* The effect of light on the moisture of soil in a saturated atmosphere may not appear large from the standpoint of vapor pressure or moisture percentage, yet when considered from the point of view of the forces set up when dried soil is connected with free water, the effect of light is enormous.

*Definition and discussion of potential.* In order to calculate these forces it will be necessary to give a general review of the meaning of potential,<sup>3</sup> the various potentials used, and the methods of calculating them.

In a singly connected, conservative force field the potential at any point is the amount of work required to move unit mass from an arbitrarily chosen point of zero potential to the point in question. If  $\mathbf{f}$  as a vector is the force on unit mass and  $d\mathbf{s}$  an infinitesimal vector along the path then the potential at a given point  $B$  will be:

$$W = \int_A^B \mathbf{f} \cdot d\mathbf{s} \quad (Q)$$

where  $A$  is the arbitrarily chosen point of zero potential. If there is no force in the field; that is, if there is equilibrium, the potential will be constant throughout.

In case more than one force field operates in a region the potential for each field and the potential due to the resultant field or total potential can all be calculated by equation (Q). The potential at a point is independent of the mass present and is the work that would be required to move unit mass from the point of zero potential to the point in question.

*Calculation of forces when potentials are known.* If the total potential is known throughout a region the net force per unit mass causing motion will be given by the negative gradient of the potential at any point. The average net force per unit mass between two points will be given by the difference in potential divided by the distance between the points and directed from high to low potential.

*Units.* In absolute units, which are used for formulae in this paper, potentials are expressed in dyne-centimeters per gram, written briefly dyne-cm./gm. Since the above units are small for some work it is convenient to express potentials in gram-centimeters per gram (gm. cm./gm.) or in cubic centimeter-atmospheres per gram (cc.-atm./gm.). The relations between these units are given by the following:

$$1 \text{ cc.-atm./gm.} = 76 \times 13.6 \text{ gm.-cm./gm.} = 1034 \text{ gm.-cm./gm.} \quad (R)$$

$$1 \text{ gm.-cm./gm.} = 980 \text{ dyne-cm./gm.} \quad (S)$$

*Potentials used.* *Gravitational potential.* In this discussion four potentials will be used frequently, they are: (a) the gravitational potential indicated by  $\phi$ , (b) the pressure potential indicated by  $\pi$ , (c) the osmotic potential indicated by  $\omega$ , and (d) the sum of these three the total potential,  $\Phi$ , given by the equation:

$$\Phi = \phi + \pi + \omega \quad (T)$$

<sup>3</sup> A complete discussion of potential functions can be obtained in *Mathematical Theory of Electricity and Magnetism*, by Jeans; in *The Theory of Electricity and Magnetism*, by Webster; in *An Introduction to Mathematical Physics*, by Houstoun; or in any other good treatise on mathematical physics.

Zero gravitational potential is taken where convenient, and in absolute units has a value of  $gh$ , where  $g$  is the acceleration of gravity and  $h$  is the distance above the level of zero gravitational potential

$$\varphi = gh \quad (U)$$

**Pressure potential.** The pressure potential is the potential due to the internal stress of the material. The pressure potential,  $\pi$ , at a point  $B$  is given by the integral.

$$\pi = \int_A^B \frac{dp}{\rho} \quad (V)$$

where  $A$  is at the arbitrarily chosen point of zero pressure potential,  $p$  is the pressure in dynes per sq. cm. and  $\rho$  is the density. When liquid water is considered,  $\rho$  is constant and equal to unity; therefore the value of the integral becomes numerically equal to  $p$ , and dimensionally equal to  $\frac{p}{\rho}$ . Ordinarily the point of zero potential is taken at zero hydrostatic pressure, making the potential zero at a free flat surface of a liquid. Since the pressure beneath the surface

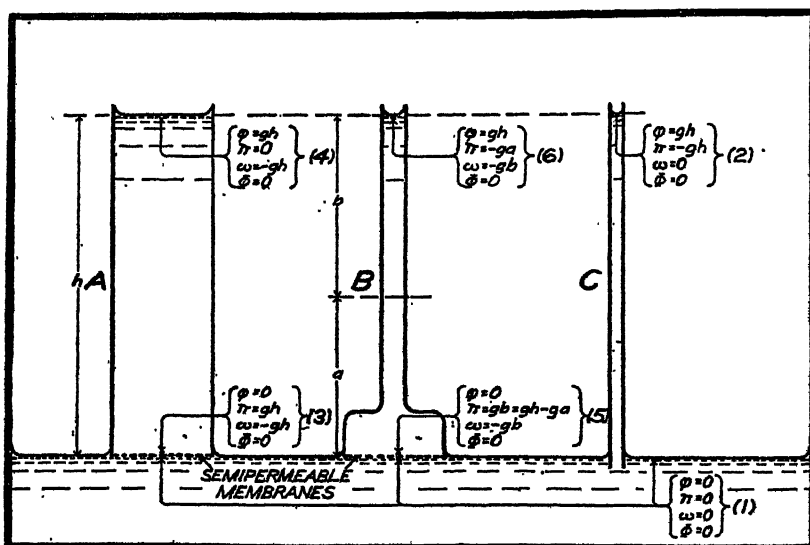


FIG. 6. DIAGRAM SHOWING THE RELATIONSHIPS BETWEEN THE VARIOUS POTENTIALS IN AN EQUILIBRIUM SYSTEM

(For description see text)

of a liquid is  $-h\rho g$ , where  $h$  is the vertical distance, positive upwards, from the free flat surface of the liquid to the point in question; and  $g$  is the acceleration of gravity. Then

$$\frac{p}{\rho} = \frac{h\rho g}{\rho} \quad (W)$$

$\pi$  is defined by equation (V) as it was by Silberstein (7), and is used because it includes both positive and negative pressures and is valid through varying densities. Some previous writers on soil moisture have used two potentials, the hydrostatic pressure potential in the regions of positive pressure and the capillary potential in regions of negative pressure. Since the only difference between the two is one of sign, the one potential  $\pi$  as defined will be used here to include both.



*Osmotic potential.* The osmotic potential,  $\omega$ , of a solution is the work necessary to transport unit mass of water through a semipermeable membrane from pure water to the solution, against osmotic forces. By definition, pure water is at zero osmotic potential. As will be shown later the osmotic potential of solutions is negative.

*Interrelations of potentials in equilibrium system.* With the aid of figure 6 the relationships between  $\pi + \omega$  and vapor pressure can be derived. In a closed vessel are the following:  $C$ , a capillary tube of such a diameter that water will rise in it to a height  $h$  above the free surface of the liquid;  $A$ , a large tube with a semipermeable membrane on the bottom and filled with a uniform solution of such strength that the liquid will rise to a height  $h$ ; and  $B$ , a capillary tube of such diameter that it will lift water to a height  $a$ , and filled with a solution of such concentration as to raise the solution a height  $b$ , with the further condition that  $a + b = h$ . This would make the top of the liquids in the three tubes the same height. The closed vessel with air removed contains a system of water, its vapor and solutions.

If the entire system is brought to isothermal equilibrium there can be no movement of liquid or vapor in cycles. Throughout the liquid phase the total potential must be constant, because if not there would be unbalanced forces causing motions in the system.  $\pi$  and  $\omega$  have already been defined as zero at the free flat pure water surface, so for convenience  $\varphi$  will also be arbitrarily chosen as zero, making the total potential  $\Phi$  zero. Since  $\Phi$  is constant, the total potential is zero throughout the liquid phase.

Since:

$$\varphi = gh \quad (U)$$

$$\pi = -gh \quad (W)$$

$$\Phi = 0 \quad (X)$$

and

$\omega$  can be calculated throughout the system.

As was previously stated at the free flat water surfaces, positions (1) (fig. 6) all the potentials are by definition zero. In the water at the top of the capillary tube, position (2),  $\omega = 0$  by definition;  $\varphi = gh$  (U); and  $\pi = -gh$  (W).

In tube  $A$  just above the membrane, position (3),  $\varphi = 0$  because it is at  $h = 0$ ;  $\pi = gh$  by (W) since the point is a distance  $h$  below the free surface of the liquid in the tube; and since  $\Phi = 0$  in the liquid,  $\omega$  must be  $-gh$ . At the surface of the liquid in the tube the pressure potential is zero,  $\varphi = gh$ , and since  $\Phi$  is zero,  $\omega = -gh$ . Since the concentration is the same at the top and bottom of the tube it would be expected that the value of  $\omega$  would be the same at both places. It can also be seen that its value is given by:

$$\omega = -gh \quad (Y)$$

where  $h$  is the height to which the solution will rise in a large tube by osmosis through a semipermeable membrane.

Just above the membrane in tube  $B$ , the gravitational potential would be zero;  $\omega = -gb$  (Y) since the solution is concentrated enough to rise to a height  $b$ ; and since the total potential is zero,  $\pi = gb = gh - ga$ , because  $a + b = h$ . At the surface of the liquid in the tube the osmotic potential would be the same as at the bottom;  $\varphi = gh$  (U); and since  $\Phi = 0$ ,  $\pi = -gh + gb = -ga$ .

It is important to note that the sum of the osmotic and pressure potentials at the top of every tube is the same and equal to  $-gh$ , and that these three surfaces are in equilibrium with the vapor at the same vapor pressure. As shown by the above where there is a combination of capillary and osmotic potentials present the following equation holds:

$$\pi + \omega = -gh \quad (Z)$$

where  $h$  is the height above the level of a free flat pure water surface in isothermal equilibrium.

*Relationship between  $\pi + \omega$  and vapor pressure.* The relationship between  $\pi + \omega$  in the liquid and the equilibrium vapor pressure can now be developed.

The pressure  $p$  of a vapor at height  $h$  above a free flat pure water surface, is given by the exponential equation:

$$p = p_0 e^{\frac{-p_{90}}{p_0} gh} \quad (A_1)$$

Where  $p_o$  and  $\rho_{vo}$  are the vapor pressure and density respectively at the free flat pure water surface,

Solving (Z) for  $h$ :

$$h = - \frac{\pi + \omega}{g} \quad (B_1)$$

Substituting value of  $h$  from (B<sub>1</sub>) in (A<sub>1</sub>):

$$p = p_o e^{(\pi + \omega) \frac{\rho_{vo}}{p_o}} \quad (C_1)$$

Transforming (C<sub>1</sub>) to logarithmic form where  $\ln$  stands for natural logarithms, that is, to the base  $e = 2.7183$ —:

$$\pi + \omega = \frac{p_o}{\rho_{vo}} \ln \frac{p}{p_o} \quad (D_1)$$

These equations are general and hold for either pressure potential or osmotic potential or both. The addition of air into the system would increase the pressure throughout by a constant amount. But since the value of  $\pi$  is defined as zero at the free flat surface of the liquid the pressure potentials referred to that surface as zero would remain the same. The other potentials would not be affected.

*Calculation of forces.* By using formula (D<sub>1</sub>) the forces set up between a dry soil and pure water can be calculated. As was shown in table 3, a difference of 0.34°C. in temperature between the soil and water would cause the soil to dry out to 98 per cent of its maximum vapor pressure. If the water is 25.0°C. its vapor pressure would be 2.378 cm. of mercury, and since the soil is in equilibrium with this water, its vapor pressure,  $p$ , would be the same. As previously stated this would be 98 per cent of its maximum vapor pressure,  $p_o$ , which would be 2.427 cm. The density of this vapor,  $\rho_{vo}$ , would be 0.000,023,50 gm. per cc. Then by (D<sub>1</sub>), since the pressures must be reduced to dynes per square centimeter:

$$\begin{aligned} \pi + \omega &= \frac{2.427 \times 13.6 \times 980}{0.000,023,50} \ln \frac{2.378 \times 13.6 \times 980}{2.427 \times 13.6 \times 980} \\ &= -27,819,900 \text{ dyne-cm./gm.} \\ &= -28,381.1 \text{ gm.-cm./gm.} \\ \pi + \omega &= -27.46 \text{ cc.-atm./gm.} \end{aligned}$$

Since in a washed soil the osmotic potential,  $\omega$ , is practically zero the value calculated above is the pressure potential,  $\pi$ . If this soil is 10 cm. from the water and is connected by a wick there would be an average net force of  $\frac{28,381}{10} = 2,838.1$  gm. for each gram of water in the wick driving it from the water to the soil. From this it is evident that ordinary laboratory light is enough to cause serious departures from mechanical equilibrium.

#### *The effect of different temperatures on equilibria*

By the use of potentials some of the temperature effects on absorption of vapor, reported by various workers under different conditions can be explained. For instance Hilgard (3) reports that the "hygroscopic coefficient" increases

with the temperature, whereas Patten and Gallagher (5) report that the amount of water vapor absorbed by a soil from a saturated atmosphere decreases as the temperature rises. These experiments were carried out under widely different conditions. Hilgard placed his soils in boxes lined with wet blotters. These were kept in a cellar, where any temperature fluctuations would be very slow. Inside of a wood box the effect of light would be almost negligible, but since in his experiments he allowed only 8 hours for absorption, equilibrium would not have been reached. This is evident from the fact that in the absorption experiments carried out by the author equilibrium had not been reached after 56 days. Since, in Hilgard's experiments, equilibrium had not been reached, the more available the water vapor was made to the soil the faster it would absorb it, and since the rate of diffusion of water vapor increases rapidly with the temperature, the higher the temperature the faster would be the diffusion of vapor to the soils and the more they would absorb in a limited time.

In contrast to this, Patten and Gallagher allowed enough time for equilibrium to be reached but they had the soils in desiccators in a thermostat heated with incandescent lamps and as a result the soils were at a higher temperature than the solutions in the bottom of the desiccator. The result would be that the soils would come to vapor equilibrium at a dryer point than if there were no light. At isothermal equilibrium the total potential of the soil and solution must be the same, for if not and if the soil and solution were connected by suitable wick there would be a motion of water. When it is remembered that the drier the soil the more negative the pressure potential, it can be seen that when soil first in isothermal equilibrium with a solution is exposed to light, the resultant drying will make the potential of the soil less than that of the solution. Thus there is a positive heat of transference from the solution to the soil. This heat of transference is positive since the potential of the soil is less than of the solution, which means that work will be done by the water in transferring itself from the solution to the soil and thus heat will be given off. By Le Chatlier's theorem of mobile equilibrium, if the temperature of the system is changed the equilibrium will be displaced in such a way as to diminish the change in temperature. This tendency to cool if the system is heated will be accomplished by distillation of water from the soil to the solution, so if the temperature is increased the soil will dry.

If in either case an isothermal equilibrium had been reached the potential of the soil and of the solution would have been the same, so there could have been no heat of transference of water in either direction. By the above theorem a change in temperature would in no way affect the equilibrium point.

Another effect would tend to increase the error made by Patten and Gallagher. At higher temperatures more heat would be required in the thermostat and thus there would be more light and a greater resultant drying.

The detailed discussion of potentials and of their relation to vapor pressure has been useful in calculating mechanical stresses caused by light, the driving

forces in soil moisture, and has given a method of determining the effect of temperature on equilibria. This latter shows that in at least the above two cases cited, the experimental results show that the workers had not reached the "hygroscopic coefficient" as they had defined it. This strengthens the conclusion previously reached that soil cannot be in isothermal equilibrium with a saturated vapor when drier than saturation.

#### SUMMARY

An unexpected drying out of wet soils was noted when they were placed in desiccators over water in a constant temperature bath.

Several absorption experiments were carried out in an apparatus designed so that it was possible to determine the amount of water absorbed at any time without opening the desiccator or removing it from the bath.

The amount of water absorbed from a saturated atmosphere when the desiccator was kept dark exceeded twice the so-called "hygroscopic coefficient." When a beam of light was turned into the desiccator all the soils dried out to a moisture content equivalent to about 84 per cent of their maximum vapor pressures. When the lights were turned off the soils began to absorb moisture again, showing that light absorbed by the soil caused the drying out noted.

A thermocouple was used to measure the difference in temperature between a soil and water in a submerged desiccator. The difference was found to be 4.5°C. when a 300 c.p. light with a reflector 50 cm. away was used, 0.5°C. when the reflector was removed, 0.16°C. due to the laboratory light during the day and within the experimental error at night.

To make sure that there could be no isothermal equilibrium between relatively dry soil and a saturated water vapor it was shown by geometry and mechanics that, at least for uniform spheres, such an equilibrium would be impossible.

A general discussion of potentials is given and four—the gravitational, pressure, osmotic, and total potentials—are defined. The relationship between the sum of the osmotic and pressure potentials in a liquid and the equilibrium vapor pressure is derived.

The forces that exist between wet and dry soils in contact are shown to be enormous, by calculating the pressure potentials from vapor pressure data. The relationship between these two functions is derived.

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# NITROGEN AVAILABILITY OF GREEN MANURES

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Maintenance and restoration of humus and nitrogen in American soils is a problem of steadily increasing importance. Because of climatic conditions American soils are especially exposed to deterioration by rapid destruction of humus. Accordingly, the necessity of incorporating organic residues into the soil deserves most careful attention.

Animal manure has always held the first place in European agriculture, whereas green manure, although used since ancient times, has served more or less as a substitute. In America, as economic conditions often favor green manuring, accurate knowledge of the results to be expected from the application of this method is highly desirable.

At present, the amount of reliable data available in this direction is not very large, and opinions differ widely concerning the extent to which the green manure nitrogen may become available to succeeding crops. The investigations herein reported were made in order to secure additional information upon this important problem. They were started in 1914, and the data presented cover a period of 10 years.<sup>1</sup>

## LITERATURE

Frequently the statement has been made that by using leguminous plants for green manure large quantities of nitrogen, equivalent to an almost seven-fold amount of nitrate, could be drawn from the air without cost. In such cases the cost of growing green manure crops has been overlooked, and furthermore, accurate tests have shown green manure nitrogen to be more or less inferior to nitrate nitrogen. There are enough valid reasons for the increased use of legumes in American agriculture to make unfounded recommendations more than superfluous.

Nitrate nitrogen is directly accessible to the roots of the cultivated plants, whereas most of the green manure nitrogen at first enters the metabolism of the soil microflora and microfauna. To what extent and how soon it will become available as plant-food depends on many variable circumstances, which are responsible for the somewhat slow and uncertain nitrogen availability of green manures. The efficiency of mineral nitrogenous fertilizers is likewise not constant, because of losses by leaching and by bacterial action. But the fluctua-

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<sup>1</sup> The work done in the field and in the greenhouse was mainly under the supervision of Messrs. F. M. Scales and N. R. Smith. Most of the analyses and calculations have been made by Messrs. A. P. Harrison and K. S. Markley. The illustrations accompanying this report were prepared by Mr. F. L. Goll. The writer is greatly indebted to his associates and assistants for their faithful collaboration.

tions and reductions are, as a rule, greater with organic manures than with ammonia and nitrate. However, the production of carbon dioxide from decaying organic residues and their manifold influences upon biochemical processes in the soil may occasionally increase the nitrogen efficiency of organic manures beyond that of mineral fertilizers.

Experimental data published before 1910 (25) indicated that the nitrogen availability of leguminous green manures may vary between 16 and 87 per cent, and that generally a return of about 50 per cent of the total nitrogen in the manured crops is to be expected. These figures are higher than those usually obtained with animal manures, wherein the nitrogen availability was found fluctuating between 10 and 40, most frequently around 25 per cent (25). More recent observations confirm and amplify these earlier findings. A number of field tests (16, 47) showed not more than 10 to 20 per cent availability of green manure nitrogen, while more frequently 30 to 40 per cent was regained in regular farm practice (17) and approximately 30 to 50 per cent in carefully conducted trials on experimental fields (40). In America higher temperatures than in Europe, heavier rains, and periods of warm weather during winter, together with the common practice of leaving the fields bare for a considerable time of the year, even where this is not necessary for conserving soil moisture for the following crops, cause, as a rule, larger losses of nitrogen not only from organic manures but also from mineral fertilizers. Accordingly, pot experiments wherein such losses are lower or absent, produce regularly higher results (43).

The same holds true in regard to nitrification tests made under laboratory conditions. In Californian soils up to 50 per cent of the total nitrogen of green manures was converted into nitrate within three weeks; Melilotus and Cowpea (*Vigna catjang*) proved to be superior to soybean (29). Experiments made in India showed within four weeks 50 per cent nitrification of the nitrogen contained in cowpea leaves and 15 per cent from the cowpea roots (20); 50 to 60 per cent of the nitrogen of *Crotalaria juncea* became mineralized within eight weeks (19). Analogous four-month tests made with green rye and green vetch in different soils (from California and from the District of Columbia) showed almost complete transformation in the case of rye—94.7 per cent—whereas the vetch gave 73.5 per cent nitrification (49).

Such results explain the slight effect of green manuring when plants rich in water and nitrogen are plowed under while the air and soil temperatures are high and a replanting of the manured field is delayed for months. The nitrate rapidly formed is easily washed away by a few heavy rains. Large quantities of rather coarse material such as almost mature rye and oats, which offer more resistance to complete decomposition, will give better results than legumes under such conditions. Humus formation and the resulting increase in the water-holding capacity of the soil are in this case of greater influence upon the succeeding crop than the amount of nitrates derived from the green manures (6, 22).

The age at which the plants, both non-legumes (28) and legumes (3) are turned under, may or may not greatly affect their nitrogen availability. Much more nitrate is to be expected from broad-leaved plants than from cereals, and the nitrification is greatly depressed if dry stems predominate in the green manures.

The comparatively high nitrification of immature material, however, is usually more than compensated by the lower weight of the young plants. The surface growth of a crop of legumes in full bloom is as a rule twice as heavy as before blooming (31). Field experiments made in Alabama (10) with hairy vetch showed for instance an increase of nitrogen in the vines from 117 to 173 pounds and in stubble and roots from 20 to 29 pounds per acre. Data secured in Delaware (35) for crimson clover gave 56 and 108 pounds nitrogen per acre in the tops and 13 and 38 pounds in the roots. Pot experiments with soybeans (24) as well as with soybeans and cowpeas (45) gave three- to four-fold increases, which are partly due to the more favorable conditions under which the plants are grown in pot experiments.

The nitrogen availability for stubble and roots is probably always much lower than that of the surface growth with the exception of the easily decomposed root nodules, whose weight is insignificant. The statement is frequently repeated in the American literature

that about one-third of the total amount of nitrogen present in a leguminous crop is contained in stubble and roots, but it must be emphasized that such high figures are more an exception than the rule. Tests made with peas in Alabama (26) gave 58 to 227 pounds nitrogen per acre for the vines, and only 3 to 14 pounds for the roots. Experiments conducted at the same station (9, 10) with crimson clover and hairy vetch showed 120 to 173 pounds nitrogen present in the tops, and 24 to 29 pounds in the roots; the analogous figures for cowpeas were 48 and 5.68, respectively (11). Almost identical results have been recorded at the Cornell Station (8). One-tenth or less of the total nitrogen was found in the soybean, cowpea, vetch, and crimson clover roots harvested at the Delaware Experiment Station (34), whereas the red clover and alfalfa roots contained one-half or three-fourths as much nitrogen as the surface growth. The latter, however, was subnormal (55 to 70 pounds nitrogen per acre). Heavier growth of alfalfa (with 122 pounds nitrogen per acre) showed one-half of the nitrogen present in the roots, and sweet clover (with 128 pounds nitrogen) approximately one-eighth (2). In old clover and alfalfa fields occasionally very conspicuous quantities of nitrogen have been found in the roots (1, 5), but such results should not be generalized (25, p. 578). This also holds true concerning some data obtained at the Cornell Station (37) with different clovers sown in August and harvested in November of the same year, viz., in crimson clover tops, 125; in the roots, 31 pounds; in red clover tops, 63; in the roots, 40 pounds; in Mammoth clover tops, 67 pounds; in the roots, 78 pounds nitrogen per acre. In field experiments conducted in Indiana at the Purdue station for four years (48) one-seventh of the total nitrogen harvested in cowpeas, and one-ninth of that in soybeans was found in the roots. Numerous observations recorded in the German literature (31, 33) indicate likewise that clover residues may contain one-third to one-half of the total nitrogen, whereas other legumes usually do not leave more than one-eighth or one-tenth of the total nitrogen in their stubble and roots, sometimes even not more than one-twentieth (41).

These data are of fundamental importance for a correct understanding of the beneficial effect exerted by the legumes upon succeeding crops. The availability of the nitrogen in the roots will rarely surpass one-third that in the tops (21); therefore, if a leguminous crop contains 150 pounds total nitrogen of which 15 to 30 pounds remain in the stubble and roots, not more than about 2 to 5 pounds nitrogen per acre will become available to the following crop from this source. Undoubtedly no conspicuous effect can be expected from this small amount of nitrogen.

How much of the legume nitrogen has been assimilated from the air, is another point upon which widely divergent estimates are recorded in the literature. Under field conditions invariably some soil nitrogen is taken up by the roots, and it is undoubtedly a mistake to calculate all the nitrogen in a leguminous crop as a clear gain in nitrogen from the air, as is sometimes done. If a soil is originally free from root-nodule bacteria and its nitrate content is very low, well inoculated legumes will draw almost all the nitrogen they need from the air, whereas uninoculated test plants will show very little development. Actual gains of 100 pounds per acre have been observed under such conditions with hairy vetch, and 140 pounds with crimson clover in experiments made in Alabama (9). Analogous tests made with alfalfa in Illinois (18) furnished up to 90 pounds per acre, with hairy vetch in Connecticut (39) approximately 70 pounds, and with different legumes in California (30) again 90 pounds per acre. Alfalfa experiments conducted in Wisconsin (13) showed that in this case 40 per cent of the nitrogen was taken from the soil and 60 per cent from the air. Other tests indicate that from 15 to 90 per cent of the total nitrogen may have been drawn from the air (5). Since, however, most soils do not furnish more than 25 to 50 pounds nitrate nitrogen to such non-leguminous crops as are generally grown in this country, and since on the other hand an average crop of legumes, even after removal of the surface growth, does not decrease, but more often increases, the supply of total nitrogen in the soil, it seems safe to assume that at least two-thirds or three-fourths of the 100 or 150 pounds nitrogen per acre harvested in a leguminous crop has come from the air. Legumes well



adapted to the particular soil and climate will almost invariably produce crops that contain as much or even more nitrogen (17; 25, p. 667; 33), although returns of 50 or 60 pounds per acre are accepted as fair averages by some authors (42).

The cost per pound nitrogen varies according to the size of the crop and to the expenses for preparation of the soil and for seed. They have been estimated at 2 to 8 cents per pound (33) for a normal crop of about 125 pounds nitrogen per acre. With 50 per cent average nitrogen availability the use of legumes for green manuring is undoubtedly more economical than that of artificial nitrogenous fertilizers, although, as a rule, it is still more advisable to use the legumes as feed, and to return the animal manure to the field whenever this is feasible.

#### METHODS

The availability of green manure nitrogen depends mainly on the following three factors:

1. Quality and quantity of the green manure.
2. Time of application and quality of the soil.
3. Kinds of crops that follow after the green manuring.

Vegetation tests, therefore, are the only means of ascertaining with fair accuracy the effect exerted by these factors.

If the nitrogen availability depended merely on the quality of the green manure plants, such a determination might be possible by chemical tests. Several methods have been recommended for this purpose. The amount of nitrogen soluble in water or in water saturated with carbon dioxide has been declared to be an index of its availability (32). Occasionally 40 or 50 per cent of the total nitrogen has been found to be soluble (32, 45). This apparent agreement with the results of other availability experiments has been accepted as proof of the correctness of that assumption. But more frequently the nitrogen solubility has been found to be much lower. Very little of the soluble nitrogen is present as ammonia, about one-half of it occurs as amino acids (15). The possibility exists that these nitrogenous compounds may be directly absorbed by the manured plants (12, 23), but since they are readily attacked by numerous soil bacteria, as a rule mineralization will take place (4). The permanganate test, used for determining the available nitrogen in commercial organic fertilizers, is likewise not applicable. Its unreliability has become more and more evident (14, 38).

Nitrification tests, on the other hand, are undoubtedly better suited for securing information upon the rapidity and the extent of the mineralization of organic nitrogen. However, the results obtained are strongly influenced by the conditions under which these tests are made. Not only the quality, but especially the quantity of the organic substances which are added to the soil is of great influence; and for analytical purposes much larger quantities always must be used in nitrification experiments than are applied in farm practice. Furthermore, soils vary considerably in their nitrifying efficiency, not only from one another, but also within themselves because of seasonal and other changes. In laboratory tests, temperature, moisture, and aeration differ more or less from those regulating field nitrification. The time factor exerts its influence, too. Therefore, in general the results of nitrification tests can not be accepted as of definite value. Improvements in this direction are quite feasible, but at present the vegetation experiment undoubtedly deserves preference.

Vegetation tests in the field are of greatest practical value, but lack of uniformity in the soil, nitrogen losses by leaching, and many other disturbing factors will always impair the reliability of the results so obtained. If the land destined for experimental purposes is repeatedly tested in advance by weighing the crops grown on the different plots with uniform treatment, the selection of an unsuitable location can be prevented. The field experiments made in connection with these investigations were all based upon the outcome of such preliminary tests.

Vegetation tests in the greenhouse are free from many of the disadvantages of field tests, but the different conditions in the two tests will influence the results.

In order to ascertain the full effect of any kind of organic manure, the experiment must be of sufficient duration. As a rule, several years are necessary for the complete mineralization of nitrogen applied in this form.

If legumes are grown on the same soil wherein the manuring effect is to be tested, the results do not demonstrate exclusively the nitrogen availability of the plants incorporated in the soil. Accordingly, the plant material used for the availability experiments was grown on field plots, harvested at different times, and applied after it had been carefully dried.

Drying of green manures, which is unavoidable in such comparative experiments as were planned, has been found to cause a reduction in their efficiency especially with single crop tests (6, 19, 36, 44, 46). But if the drying is done slowly, if all losses of leaves are avoided, and if the experiments are of long duration, the final results are not seriously affected. A few comparative tests made with corn, kafir, and milo, manured with fresh and dried pea tops of the same nitrogen content gave lower weights but larger nitrogen returns from the dried green manure.

PEA VINES APPLIED	INCREASE IN FRESH WEIGHT			INCREASE IN NITROGEN EFFICIENCY		
	Corn	Kafir	Milo	Corn	Kafir	Milo
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Fresh.....	63	70	55	56	3	7
Dried.....	57	55	33	50	23	40

The green manures used in the pot experiments were applied in quantities of 30, 10, and 3.3 gm. dry weight, respectively. These amounts are approximately equivalent to  $4\frac{1}{2}$ ,  $1\frac{1}{2}$ , and  $\frac{1}{2}$  tons air-dry substance with 225, 75, and 25 pounds nitrogen per acre, and represent a large, a medium, and a small application of green manure. The smallest quantity represents practically that usually left on the field as stubble after a legume has been cut for hay.

The various legumes had been taken from the field before the buds opened (young), when one half of the growth was in full bloom (medium), and after blooming when the pods had been partly formed (old). Ripe cowpeas with mature seed, before and after they had been exposed to frost, were included in the test.

Two different soils were used: a heavy clay subsoil of low activity and low in humus (0.103 per cent N), and a very fertile greenhouse soil of fairly high activity and well enriched with humus and nitrogen (0.143 per cent N). Phosphate and potash were adequate in both soils, and the original reaction was close to neutral. Nevertheless, in order to prevent any possible lack of lime, phosphate, and potash that might have occurred after a number of crops had been taken, alternating applications of 2 gm. monopotassium-phosphate, and 5 gm. calcium carbonate per pot were applied to every second planting.

For each series 296 galvanized pails were used. Every pot was filled with 5 pounds gravel and 20 pounds soil. Two strong glass tubes, reaching from the top down into the layer of gravel, assured proper aeration. The plan for the pot tests is shown in table 1.

After the pots had been filled with gravel and soil, in order to ascertain the uniformity of the soils a crop of unmanured buckwheat was first raised on all pails. The results of this test were quite satisfactory on the poor soil, but the rich soil, with its larger amount of available humus nitrogen, gave less consistent figures as is shown by the data for the mean probable error in tables 2 and 3. Renewed mixing of the contents of a few pails which gave exceptionally high or low crops eliminated these inequalities. Accordingly, the mean

TABLE 1  
*Arrangement of pot tests*

POOR SOIL (NORTH HOUSE)				RICH SOIL (SOUTH HOUSE)				
Manures	N	Per pot		Manures	N	Per pot		Pot numbers
		Dry	mgm.			Dry	mgm.	
None.....	.....	.....	.....	None.....	.....	.....	.....	151-851
Pea, medium.....	2.85	30.0	855.0	Sweet Clover, young.....	2.54	30.0	762.0	152-852
		10.0	285.0			10.0	254.0	153-853
		3.3	94.1			3.3	83.8	154-854
White Lupin, young.....	2.37	30.0	711.0	Sweet Clover, medium.....	2.68	30.0	804.0	155-855
		10.0	237.0			10.0	268.0	156-856
		3.3	78.2			3.3	88.4	157-857
None.....	.....	.....	.....	None.....	.....	.....	.....	158-858
White Lupin, medium.....	1.78	30.0	534.0	Sweet Clover, old.....	2.13	30.0	639.0	159-859
		10.0	178.0			10.0	213.0	160-860
		3.3	58.7			3.3	70.3	161-861
White Lupin, old.....	1.63	30.0	489.0	Cowpea, young.....	3.54	10.0	354.0	162-862
		10.0	163.0			3.3	116.8	163-863
		3.3	53.8			10.0	271.0	164-864
None.....	.....	.....	.....	Cowpea, medium.....	2.71	3.3	89.4	165-865

Hairy Vetch, young.....	3.27	30.0	981.0	116-816	None.....	....	....	166-866
		10.0	327.0	117-817				
		3.3	107.9	118-818				
Hairy Vetch, medium.....	2.71	30.0	813.0	119-819	Cowpea, old.....	2.18	30.0 10.0 3.3	167-867 168-868 169-869
		10.0	271.0	120-820				
		3.3	89.4	121-821				
Horse Bean, young.....	2.97	30.0	297.0	122-822	Cowpea, ripe.....	2.00	30.0 10.0 3.3	170-870 171-871 172-872
		10.0	98.0	123-823				
		3.3	.....	.....				
None.....	....	....	....	124-824	None.....	....	....	173-873
		....	....	....				
		....	....	....				
Horse Bean, old.....	2.91	30.0	873.0	125-825	Cowpea, frozen.....	1.97	30.0 10.0 3.3	174-874 175-875 176-876
		10.0	291.0	126-826				
		3.3	96.0	127-827				
Yellow Lupin, young.....	3.00	30.0	900.0	128-828	Soybean, young.....	3.28	30.0 10.0 3.3	177-877 178-878 179-879
		10.0	300.0	129-829				
		3.3	99.0	130-830				
None.....	....	....	....	131-831	None.....	....	....	180-880
		....	....	....				
		....	....	....				
Yellow Lupin, medium.....	2.01	30.0	603.0	132-832	Soybean, medium.....	2.32	30.0 10.0 3.3	181-881 182-882 183-883
		10.0	201.0	133-833				
		3.3	66.3	134-834				
Yellow Lupin, old.....	1.27	30.0	381.0	135-835	Soybean, old.....	2.15	30.0 10.0 3.3	184-884 185-885 186-886
		10.0	127.0	136-836				
		3.3	41.9	137-837				
					None.....	....	....	187-887

TABLE 2  
*Mean probable error\* and deviations from average checks. Fresh weights on poor soil*

MANURES AFTER BUCKWHEAT	MEAN PERCENTAGE PROBABLE ERROR, SINGLE CROPS										ALL CROPS	
	Buck-wheat	Corn	Corn	Oats	Cotton	Rye	Flax	Millet	Buck-wheat	Corn	Prob-able error	Devia-tion from average check
	gm.											per cent
None.....	3.6	3.4	5.2	3.4	5.1	5.6	3.5	8.7	6.7	2.5	4.8	2.1
Pea, medium.....	30.0	2.8	5.5	3.4	3.0	4.4	2.0	9.6	5.1	2.4	4.2	46.5
	10.0	2.7	6.5	4.4	2.9	6.1	2.6	5.6	3.9	2.9	4.3	21.8
	3.3	3.3	5.4	4.0	3.6	5.1	3.1	7.4	5.4	2.6	4.6	9.6
White Lupin, young.....	30.0	4.5	6.0	2.5	5.4	5.3	3.5	9.3	3.9	2.3	4.8	36.2
	10.0	4.5	5.6	2.9	6.5	3.5	2.8	6.5	5.3	4.4	4.4	14.4
	3.3	3.7	6.6	3.1	5.2	4.7	2.3	7.4	6.3	3.2	4.6	6.8
None.....	2.7	4.5	6.5	5.2	5.1	6.4	3.1	6.6	5.0	1.9	4.7	-2.0
White Lupin, medium.....	30.0	2.9	4.4	4.0	2.7	3.8	4.0	6.8	5.2	2.1	3.8	24.0
	10.0	4.0	5.1	2.7	3.4	4.0	6.1	11.7	4.3	3.7	4.9	12.5
	3.3	3.3	5.9	3.1	5.1	4.2	0.6	8.0	4.6	2.2	4.0	6.2
White Lupin, old.....	30.0	2.4	2.9	3.6	3.7	3.5	4.5	8.9	4.0	3.5	4.0	23.2
	10.0	3.2	8.5	6.4	4.5	3.3	0.6	5.6	2.2	2.4	3.8	6.3
	3.3	3.5	4.8	2.4	4.6	5.7	3.9	6.3	3.0	1.8	3.8	4.9
None.....	3.3	3.2	5.8	3.0	3.8	4.2	4.2	9.7	6.1	2.7	4.6	1.5

Hairy Vetch, young.....	30.0	5.7	3.4	4.2	3.2	5.0	4.9	3.1	8.3	1.5	3.5	4.3	43.3
	10.0	3.8	1.6	7.5	2.3	4.3	8.4	3.2	4.5	4.1	2.7	4.2	20.6
	3.3	4.6	2.5	6.1	3.4	5.7	6.1	2.9	5.3	4.0	3.0	4.4	11.5
Hairy Vetch, medium.....	30.0	2.3	1.7	6.8	4.1	5.3	5.6	3.4	9.1	3.9	3.8	4.6	36.8
	10.0	5.2	1.3	5.2	5.2	4.7	4.0	2.9	8.1	2.8	3.2	4.3	16.0
	3.3	3.6	2.2	6.6	2.7	6.2	6.8	3.9	4.8	4.9	2.4	4.4	3.9
Horse Bean, young.....	10.0	5.2	2.4	4.7	3.2	5.8	5.1	5.3	9.6	3.5	2.6	4.7	13.0
	3.3	3.9	3.9	6.8	1.8	4.0	4.3	3.7	4.9	2.8	2.7	3.9	4.9
	.....	3.0	2.9	4.5	3.1	5.8	5.7	5.7	5.1	5.9	2.9	4.5	-0.8
Horse Bean, old.....	30.0	3.7	3.6	5.2	1.6	2.8	3.6	3.6	10.1	3.5	1.7	3.9	20.2
	10.0	4.0	2.2	4.3	3.7	4.4	4.6	5.6	5.4	3.9	2.2	4.0	11.5
	3.3	3.8	1.9	8.2	5.5	5.1	3.5	4.2	5.0	5.2	2.9	4.5	1.3
Yellow Lupin, young.....	30.0	4.3	1.9	7.7	4.2	4.5	4.2	4.2	6.0	2.4	3.2	4.3	32.6
	10.0	3.6	1.8	3.9	2.5	4.3	3.1	2.8	5.7	4.0	2.6	3.4	11.3
	3.3	2.4	2.3	4.7	3.3	6.0	3.6	0.6	6.4	4.4	1.2	3.5	-1.6
None.....	.....	2.8	2.1	8.6	1.9	5.2	2.2	2.9	7.2	4.4	1.9	3.9	-1.4
	30.0	5.2	2.0	3.1	1.6	3.9	2.0	2.4	7.3	5.0	3.7	3.6	28.9
	10.0	4.1	2.1	4.0	3.4	4.3	1.5	2.2	7.0	3.9	4.1	3.7	8.9
Yellow Lupin, medium.....	3.3	3.6	2.7	5.3	2.3	6.0	3.0	0.6	6.2	4.6	3.1	3.7	2.1
	30.0	3.3	2.1	3.7	2.5	5.5	4.6	3.5	8.4	2.9	3.6	4.0	2.5
	10.0	4.3	2.5	5.9	2.7	6.0	4.5	0.6	5.1	3.4	3.4	3.3	-0.8
Yellow Lupin, old.....	3.3	2.9	3.9	6.9	2.8	6.6	4.2	4.5	8.0	4.4	1.8	4.6	-5.8
	.....	3.1	3.6	6.1	3.7	4.7	5.0	3.9	7.5	5.6	2.4	4.6	....
	Averages.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

$$* (r = 0.6745 \sqrt{\frac{\sum(d)^2}{n}})$$



Cowpea, old.....	30.0	5.4	2.9	3.3	3.6	2.2	2.8	3.2	3.3	10.2
	10.0	6.1	3.8	4.4	3.7	1.5	3.1	2.9	3.6	6.2
	3.3	6.1	1.8	4.3	2.9	3.9	2.5	3.0	3.5	4.8
Cowpea, ripe.....	30.0	3.8	3.7	3.9	3.2	4.8	1.6	1.0	3.1	9.8
	10.0	6.4	2.3	2.1	2.6	3.0	2.4	1.8	2.9	3.5
	3.3	5.4	7.2	3.1	3.8	3.6	2.7	1.9	4.0	-1.3
None.....	....	6.9	5.0	2.3	2.8	2.6	4.3	3.7	3.9	-0.2
Cowpea, frozen.....	30.0	7.0	6.8	3.9	2.7	3.1	1.8	3.2	4.1	7.2
	10.0	4.9	7.6	3.4	2.9	3.9	1.5	1.6	3.7	1.3
	3.3	4.0	1.5	2.8	2.4	3.1	2.9	2.5	2.7	3.4
Soybean, young.....	30.0	4.8	4.2	3.1	2.8	2.7	1.9	3.6	3.3	16.8
	10.0	5.1	4.2	2.7	3.0	2.4	2.0	2.7	3.2	6.2
	3.3	3.9	1.2	2.5	2.6	3.2	3.0	2.8	2.7	5.7
None.....	....	5.2	6.2	2.9	1.7	2.6	2.5	3.6	3.5	-0.4
Soybean, medium.....	30.0	4.7	6.2	3.3	3.5	3.4	2.5	2.7	3.8	6.2
	10.0	2.7	2.9	3.8	1.7	3.3	2.5	3.1	2.9	6.1
	3.3	3.4	3.6	2.9	2.1	3.0	3.5	3.7	3.2	4.9
Soybean, old.....	30.0	4.7	2.7	3.5	2.7	3.0	1.3	1.3	2.7	11.5
	10.0	5.7	4.5	3.4	4.8	4.6	2.9	3.4	4.2	6.1
	3.3	4.6	2.8	2.8	3.9	2.6	2.4	3.2	3.2	2.7
None.....	....	5.3	2.7	4.5	2.4	1.7	4.5	2.7	3.4	-0.9
Averages.....	5.4	3.6	3.2	1.9	2.3	3.0	2.6	3.1	....	....

$$* \left( r = 0.6745 \sqrt{\frac{\sum(d)^2}{n(n-1)}} \right)$$



probable error calculated for the following crops was the same for the rich and poor soils. The differences shown for the various crops were mainly due to the higher or lower weight of the plants grown.

The final data are always based on the results obtained with sets of 8 pots arranged in rows across the full width of the house. For exact experiments this procedure is of special importance. Frequently vegetation tests in greenhouses are made with duplicate or triplicate sets (7); however, the frequency curves shown in figure 1 for the first buckwheat crop raised on the poor soil demonstrate beyond doubt that accurate results are not to be expected unless the pots receiving the same treatment are evenly distributed over the full width of the house. Even the curves for 6 rows west or 6 rows east show marked deviations from the true average (60 gm.), although 4 of them are identical in both cases.

In the nitrogen availability experiments 1 row of 8 pots was always used for determining the effect of one kind of manuring, but after the check rows, which were scattered throughout.

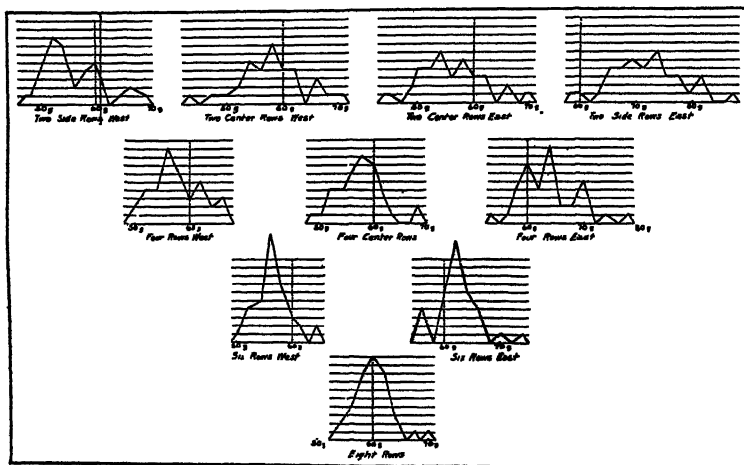


FIG. 1. FLUCTUATIONS OF FRESH WEIGHTS (BUCKWHEAT) HARVESTED IN 2 TO 8 ROWS OF 37 POTS EACH, PLACED LENGTHWISE IN THE HOUSE

Most of the pots in the west-side rows produced smaller crops than those in the east-side rows.

the house, had shown variations in crop yield because of small differences in temperature and ventilation, it seemed preferable to increase the number of parallel tests. Therefore, in subsequent experiments on crop succession, not less than 32 tests were always made in each case, that is, 4 rows of 8 equally treated pots were evenly distributed throughout the house.

Table 4 shows the fluctuations in the fresh weight of the check rows in the first set of experiments. The final figure for each row shows that these slight variations do not materially affect the results obtained with different treatments. Nevertheless, the checking system adopted for the subsequent experiments is undoubtedly preferable, and its use is to be recommended whenever long time tests are planned.

The pots were first watered by daily adjusting their weight on a balance, but it soon became evident that the results did not justify this cumbersome procedure. The desired equality in moisture was no better assured than by careful spraying by well trained men. Occasionally, overwatering will occur, and the total nitrogen balance of the pots of poor

soil proves that in this case nitrogen losses have happened, probably by denitrification in the lower layers of the heavy clay, but again these disturbances by no means impair the

TABLE 4  
*Deviations in check rows, fresh weights*

	AVERAGE CHECK	DEVIATIONS IN ROWS					
		North—Center—South					
<i>North House poor soil</i>							
	<i>gm.</i>						
Buckwheat.....	60.4	+7.1	-3.9	-6.4	-1.4	+3.6	
Corn.....	122.0	-4.0	-4.0	+7.0	+3.0	-2.0	
Corn.....	24.8	+1.4	+1.2	+2.4	-0.4	-4.4	
Oats.....	62.0	+1.1	-2.8	±0	-2.0	+3.8	
Cotton.....	18.3	-1.7	+0.2	+1.7	+0.7	-1.2	
Rye.....	19.0	+0.1	+2.1	-1.4	-0.4	-0.4	
Flax.....	10.0	-0.3	+0.9	-0.1	-0.9	-0.2	
Millet.....	25.5	+6.5	-1.5	-4.9	+3.7	-3.8	
Buckwheat.....	37.3	+2.9	+1.6	+2.5	-3.0	-4.1	
Corn.....	78.4	-2.0	-4.1	+6.9	-2.1	+1.5	
Wheat.....	8.4	+0.9	+0.1	+0.5	-1.0	-0.4	
Total.....	{ ..... .....	+18.0	+6.1	+20.5	+7.4	+8.9	
		-8.0	-16.3	-12.8	-11.2	-16.5	
All crops <i>gm.</i> .....	466.1	+10.0	-10.2	+7.7	-3.8	-7.6	
All crops <i>per cent.</i> .....	100.0	+2.1	-2.2	+1.5	-0.8	-1.4	
<i>South House rich soil</i>							
Buckwheat.....	26.5	+6.2	-1.1	-1.6	-6.4	-2.8	+6.9
Corn.....	316.0	+2.0	+4.0	-1.0	+2.0	-6.0	-3.0
Oats.....	111.0	-5.0	-3.0	+3.0	-4.0	+2.0	+3.0
Rye.....	89.0	-4.0	+4.0	+4.0	+2.0	±0	-9.0
Millet.....	61.0	-4.0	+2.0	-1.0	+5.0	+1.0	+1.0
Buckwheat.....	42.2	+1.3	-2.2	+1.3	-0.2	+1.8	-1.7
Corn.....	62.2	+7.8	-1.2	-3.8	-0.2	+0.8	-2.8
Wheat.....	42.0	+2.0	±0	.....	.....	±0	-1.0
Total.....	{ ..... .....	+19.3	+10.0	+8.3	+9.0	+5.6	+10.9
		-13.0	-7.5	-7.4	-10.8	-8.8	-17.5
All crops <i>gm.</i> .....	749.9	+6.3	+2.5	+0.9	-1.8	-3.2	-6.6
All crops <i>per cent.</i> .....	100.0	+0.8	+0.3	+0.1	-0.2	-0.4	-0.9

results to such an extent as would be the case with prolonged periods of wet weather in field experiments.

The temperatures in the greenhouse varied to some extent according to the season, as shown in table 5.

During the hottest part of the summer the experiments were usually stopped temporarily,

and the pots were kept dry so that practically no changes occurred in the nitrogen content of the soils.

After the fresh and dry weights of the crops (single pots and rows of 8) had been taken, the total nitrogen for every row of 8 pots was determined by a modified Kjeldahl method with boric acid in the receiving flasks. It proved especially useful for the prompt disposal of the very large number of digestions and distillations that had to be made (27).

TABLE 5  
*Air temperatures in the greenhouse*

	MINIMA			MAXIMA			MEAN TEMPER- ATURES
	Lowest	Highest	Average	Lowest	Highest	Average	
	°F.	°F.	°F.	°F.	°F.	°F.	°F.
January-March.....	42	67	56	62	81	71	64
March-May.....	44	66	57	61	110	84	71
June-July.....	50	76	65	73	112	96	81
August-September.....	41	68	60	70	110	89	75
October-December.....	41	66	53	59	99	77	65

#### EXPERIMENTAL RESULTS

The plants used as manures in the greenhouse experiments were grown together with a number of other legumes on small field plots at Arlington Farm. Table 6 shows the weights of the air-dried matter and of the total nitrogen calculated in pounds per acre which were harvested in 1915 and 1916.

The data recorded demonstrate clearly that not more than three or four legumes—cowpea, soybean, hairy vetch, and red clover in its second year—have made a really satisfactory growth under the prevailing soil and climate conditions. Field tests continued on the same area for 10 years have confirmed these results, with the exception that red clover has not proved sufficiently reliable. Drought and winter killing have caused about 50 per cent failures. Since only a heavy growth of legumes will benefit the succeeding crops to the fullest extent, such preliminary tests are much to be recommended in view of the widely varying field conditions. No legume can be considered well adapted to a locality if it contains less than 100 pounds nitrogen per acre after full development has been reached.

After the buckwheat test crop was harvested in the greenhouse the pots received different kinds and different quantities of dried and ground legumes as shown in table 1. On poor soil, 10 crops were raised within 4 years, on rich soil, 7 crops within 3 years. The data obtained on fresh weight, dry weight, and nitrogen harvested in the different crops are given in tables 7 to 12. Every figure represents the average of 8 single tests. Percentage calculations on crop increases and nitrogen availability are included.<sup>2</sup>

<sup>2</sup> The figures for the last crop are incomplete, because some of the pots were used for another experiment. The manuring effect had practically reached its end, and the last wheat crop was mainly raised to ascertain this fact conclusively.

TABLE 6  
*Leguminous crops at different ages*  
 Field tests in 1915 and 1916

	AIR-DRIED SUBSTANCE, POUNDS PER ACRE						NITROGEN, POUNDS PER ACRE					
	Young		Medium		Old		Young		Medium		Old	
	1915	1916	1915	1916	1915	1916	1915	1916	1915	1916	1915	1916
Alsike.....	1,590	.....	1,923	1,280	.....	2,051	37.9	.....	44.3	38.4	.....	.....
Beggarweed.....	726	770	.....	.....	1,676	1,689	20.5	21.8	.....	.....	28.5	28.7
Berseem.....	.....	.....	705	.....	1,410	.....	.....	.....	20.2	.....	32.4	.....
Blue Lupin.....	.....	.....	2,217	.....	.....	.....	.....	.....	62.3	.....	.....	.....
Chick pea.....	.....	.....	1,035	446	1,955	.....	.....	.....	35.6	11.9	45.4	.....
Cowpea.....	768	.....	1,920	5,357	5,397	11,032	27.2	.....	77.6	145.2	140.3	240.5
Crimson Clover.....	510	.....	2,420	.....	.....	.....	17.1	.....	67.3	.....	.....	.....
Fengreek.....	.....	.....	.....	319	.....	.....	.....	.....	.....	11.0	.....	.....
Field Pea.....	.....	288	991	1,263	.....	1,579	.....	12.1	28.2	44.8	.....	42.3
Hairy Vetch.....	1,930	2,052	6,017	4,461	.....	4,103	63.1	59.5	163.0	132.0	.....	102.8
Horse Bean.....	454	585	.....	.....	1,451	1,964	13.5	22.2	.....	.....	42.2	53.4
Japan Clover.....	.....	.....	.....	2,812	.....	.....	.....	.....	.....	61.3	.....	.....
Peanut.....	.....	.....	.....	654	.....	3,461	.....	.....	.....	21.7	.....	86.5
Red Clover.....	.....	1,284	.....	2,799	3,390	4,800	.....	30.5	.....	68.2	77.9	129.6
Sainfoin.....	.....	.....	.....	640	.....	.....	.....	.....	.....	11.0	.....	.....
Seed Vetch.....	.....	335	.....	1,229	.....	.....	.....	12.1	.....	38.5	.....	.....
Soybean.....	648	2,400	4,188	5,760	7,778	9,095	21.3	50.4	82.1	133.6	127.6	195.5
Sweet Clover.....	624	.....	1,110	2,180	2,560	.....	15.9	.....	29.8	43.2	50.0	.....
Velvet Bean.....	.....	2,154	4,800	.....	8,559	.....	.....	46.7	115.2	.....	196.0	.....
White Clover.....	.....	705	.....	855	.....	.....	.....	23.2	.....	24.8	.....	.....
White Lupin.....	1,110	833	3,856	1,250	2,204	.....	26.3	22.8	68.6	32.8	35.9	.....
Yellow Lupin.....	479	.....	1,293	.....	1,116	.....	14.4	.....	26.0	.....	19.8	.....
Yellow Trefoil.....	.....	.....	876	1,020	.....	.....	.....	.....	24.4	27.8	.....	.....

TABLE 7

*Fresh weights on poor soil*

Averages grams per pot

MANURES	1	2	3	4	5	6	7	8	9	4-9	10
	CORN	CORN	OATS	COTTON	EYE	FLAX	MILLET	BUCK- WHEAT	CORN	INCREASE	WHEAT
										<i>per cent</i>	
None.....	118	26.2	63.1	16.6	18.1	9.5	33.4	38.0	76.4	(+3.0)	9.3
Pea, medium.....	30.0	233	47.5	19.3	24.3	11.1	44.9	42.5	88.0	25.3	10.9
	10.0	188	33.4	18.2	21.5	9.2	31.0	35.5	81.6	7.0	....
	3.3	151	23.2	16.1	19.4	9.1	28.0	39.0	79.6	4.0	....
White Lupin, young.....	30.0	201	50.6	18.2	24.6	10.4	32.0	40.4	88.3	16.3	10.8
	10.0	152	31.8	18.5	19.6	10.0	31.9	34.6	80.5	6.0	....
	3.3	128	32.8	19.8	20.4	9.2	27.0	37.3	78.9	5.1	....
None.....	118	26.0	59.2	18.5	20.1	10.1	25.4	36.7	74.3	(+0.5)	8.5
White Lupin, medium.....	30.0	183	38.5	20.0	21.0	10.6	26.1	38.5	84.4	14.5	9.4
	10.0	147	31.4	20.1	18.3	10.3	23.8	36.0	78.3	1.4	....
	3.3	136	26.5	19.8	16.6	9.7	24.4	36.0	81.0	1.8	....
White Lupin, old.....	30.0	175	38.7	21.2	19.6	10.6	26.2	40.8	86.6	11.5	9.6
	10.0	135	24.4	20.0	17.0	10.2	25.8	39.0	84.9	7.0	....
	3.3	129	26.6	18.4	17.7	9.6	27.1	38.5	82.3	5.1	....
None.....	129	27.2	62.0	20.3	16.6	9.1	22.0	37.6	85.3	(-1.2)	8.9
Hairy Vetch, young.....	30.0	234	57.2	21.8	20.4	11.2	27.1	42.8	86.3	13.5	9.1
	10.0	185	31.8	19.4	19.9	9.9	23.2	43.6	82.6	8.0	....
	3.3	151	24.9	20.1	17.0	9.5	20.1	41.1	84.9	4.7	....

Hairy Vetch, medium.....	30.0	227	43.9	81.2	72.2	22.5	19.7	11.5	30.1	42.8	91.3	18.5	9.1
	10.0	157	31.0	75.7	28.0	21.6	19.9	10.0	24.4	42.1	86.1	11.2	8.5
	3.3	135	24.6	62.0	7.0	21.0	17.7	9.9	23.1	36.6	83.0	4.0	8.1
Horse Bean, young.....	10.0	158	31.3	72.0	26.8	19.8	18.2	10.1	25.6	38.1	81.5	5.0	8.0
	3.3	137	26.6	64.0	10.0	18.8	16.6	9.6	26.6	35.5	81.3	2.4	8.0
	.....	125	24.4	60.0	(+0.8)	19.0	17.6	8.3	30.6	32.1	76.3	(-0.1)	7.4
Horse Bean, old.....	30.0	172	38.9	80.6	41.9	20.4	19.9	10.4	29.3	38.1	80.9	8.2	8.0
	10.0	153	30.4	69.4	27.5	18.6	18.1	9.8	25.6	33.6	84.0	3.0	7.5
	3.3	133	21.6	67.2	7.0	18.4	17.7	9.1	26.9	33.6	74.4	-2.2	7.6
Yellow Lupin, young.....	30.0	214	39.8	87.0	66.6	18.9	20.0	9.9	24.4	35.8	96.6	17.5	9.0
	10.0	163	26.9	68.3	25.3	17.7	18.2	10.3	28.5	34.8	81.5	3.8	7.9
	3.3	127	21.8	59.6	0.4	17.1	17.3	9.5	22.4	35.5	78.1	-2.3	7.5
None.....	.....	120	20.4	65.8	(-0.7)	17.1	17.6	9.0	23.1	31.0	79.9	(-3.5)	8.0
	30.0	186	40.0	88.0	53.2	19.9	23.8	10.1	28.0	37.4	90.5	13.0	8.9
	10.0	142	28.1	65.4	13.9	19.7	21.2	10.1	26.8	35.1	82.5	0.8	7.9
Yellow Lupin, medium.....	3.3	126	24.0	61.9	2.1	22.1	18.3	10.1	26.0	35.3	81.4	5.0	7.9
	30.0	110	31.2	76.0	4.7	21.2	21.2	9.7	26.7	34.4	79.5	4.7	8.6
	10.0	109	24.1	69.8	-2.4	21.5	19.0	10.0	30.8	36.4	73.6	3.9	8.5
Yellow Lupin, old.....	3.3	104	21.8	66.6	-7.1	19.5	19.0	9.9	26.8	34.4	70.8	-2.0	9.0
Average checks.....		122	24.8	62.0	.....	18.3	18.0	9.2	26.9	35.1	78.4	....	8.4

TABLE 8  
*Fresh weights on rich soil*  
Averages grams per pot

MANURES	1 CORN	1 INCREASE <i>per cent</i> (+0.7)	2 OATS	3 RYE	4 MILLET	5 BUCK- WHEAT	6 CORN	2-6 INCREASE <i>per cent</i> (-1.2)	7 WHEAT
None.....	318		106	85	57	43.5	70		44
Sweet Clover, young.....	396	26.7	108	99	66	43.5	67	5.8	44
	372	18.7	102	91	59	43.0	68	-2.1	..
	334	6.0	109	88	61	42.0	67	0.4	..
Sweet Clover, medium.....	403	29.0	110	99	67	42.5	65	5.0	..
	368	14.0	98	95	66	42.0	64	0.1	36
	339	7.7	106	95	62	44.5	64	1.7	39
None.....	320	(+1.3)	108	93	63	40.0	61	(±0)	42
Sweet Clover, old.....	385	23.0	115	100	62	44.5	61	4.8	..
	359	14.3	112	96	64	42.5	63	3.6	..
	346	10.0	108	101	62	42.0	63	2.9	..
Cowpea, young.....	366	16.7	103	103	62	43.5	64	2.7	44
	349	11.0	106	100	60	44.5	61	1.7	41
Cowpea, medium.....	353	12.3	112	94	63	46.5	65	4.2	45
	347	10.3	110	94	65	43.5	61	2.2	..
None.....	315	(-0.3)	144	93	60	43.5	58	(+1.1)	..

Cowpea, old.....	30.0	366	16.7	112	110	71	44.5	61	9.2	..
	10.0	359	14.3	110	94	71	44.0	61	4.1	39
	3.3	337	7.0	112	97	65	45.5	56	2.7	41
Cowpea, ripe.....	30.0	356	13.3	125	101	66	48.0	67	11.6	45
	10.0	338	7.7	112	94	65	44.5	65	4.2	..
	3.3	305	-3.7	120	91	65	45.0	62	4.9	..
None.....	....	318	(+0.7)	107	91	66	42.0	62	(-0.4)	..
Cowpea, frozen.....	30.0	331	5.0	113	99	73	43.5	60	6.4	41
	10.0	328	4.0	114	87	68	43.0	65	3.2	41
	3.3	305	-3.7	112	91	64	45.0	66	3.5	41
Soybean, young.....	30.0	418	34.0	112	105	73	44.0	63	8.7	..
	10.0	374	19.3	106	91	64	41.5	65	0.6	..
	3.3	362	15.3	113	89	65	43.5	63	2.2	..
None.....	....	310	(-2.0)	113	89	62	44.0	63	(+1.6)	42
Soybean, medium.....	30.0	380	21.3	116	99	68	44.5	68	8.3	44
	10.0	359	14.3	105	100	65	42.5	64	3.1	43
	3.3	347	10.3	109	103	62	42.0	64	4.0	..
Soybean, old.....	30.0	378	17.3	112	95	73	44.0	69	7.6	..
	10.0	352	12.0	111	88	70	44.5	63	3.1	..
	3.3	343	9.0	104	86	69	43.5	65	0.6	44
None.....	....	313	(-1.0)	116	80	62	40.5	59	(-2.2)	41
Average checks.....										42
		316	....	111	89	61	42.2	62.2	....	



TABLE 9

*Dry weights on poor soil*

Averages grams per pot

MANURES	1 CORN	2 CORN	3 OATS	1-3 INCREASE <i>per cent</i>	4 COTTON	5 RYE	6 FLAX	7 MILLET	8 BUCK- WHEAT	9 CORN	4-9 INCREASE <i>per cent</i>	10 WHEAT
None.....	19.2	4.0	15.4	(-0.8)	3.8	5.1	1.9	16.1	5.9	21.6	(+1.1)	2.2
Pea, medium.....	30.0	38.6	6.7	76.4	5.3	6.7	2.3	20.8	7.0	25.5	7.2	2.5
	10.0	33.2	4.4	43.4	4.8	6.3	2.1	14.4	5.1	23.5	1.0	...
	3.3	26.4	3.3	16.8	20.1	5.3	1.9	12.8	5.4	23.3	0.4	...
White Lupin, young.....	30.0	33.5	7.3	60.1	4.3	7.5	2.1	15.0	5.8	25.4	3.9	2.4
	10.0	24.8	4.4	18.2	4.6	5.8	2.0	16.0	5.1	23.4	2.5	...
	3.3	20.7	4.9	17.4	4.8	6.0	1.9	11.8	5.7	23.0	0	...
None.....	18.9	3.8	15.1	(-2.9)	4.7	5.0	2.1	11.5	5.4	22.5	(+0.5)	1.9
White Lupin, medium.....	30.0	29.8	5.7	18.8	5.5	6.1	2.1	11.1	5.7	23.8	1.0	2.2
	10.0	25.4	4.7	19.2	5.3	5.4	2.0	9.1	4.9	21.8	-1.8	...
	3.3	22.7	4.0	17.0	4.9	5.3	2.0	12.6	4.7	23.4	0.4	...
White Lupin, old.....	30.0	29.1	5.4	19.2	5.7	5.8	2.1	11.5	6.1	24.9	1.9	2.1
	10.0	21.4	3.9	17.9	5.0	5.1	2.0	11.9	5.5	24.5	-1.6	...
	3.3	20.0	3.9	16.3	5.0	5.2	1.9	11.5	5.6	24.0	0.7	...
None.....	19.4	3.9	15.0	(-1.6)	5.1	4.9	1.8	10.4	5.9	24.0	(+0.1)	2.1
Hairy Vetch, young.....	30.0	35.5	9.2	72.0	5.7	6.6	2.3	12.0	6.6	25.1	3.0	2.4
	10.0	33.2	4.4	42.4	4.9	5.8	2.1	10.4	6.6	23.5	0.6	...
	3.3	23.3	3.6	18.2	5.1	5.0	2.0	9.0	6.6	25.1	0.3	...

Hairy Vetch, medium.....	30.0	35.7	6.8	19.9	61.1	6.0	5.8	2.2	14.9	7.1	26.0	4.9	2.2
	10.0	26.9	4.4	18.3	30.4	5.5	6.1	2.2	10.8	6.8	25.0	2.1	2.0
	3.3	20.5	3.8	14.7	0.3	5.2	5.4	2.1	9.9	6.0	24.9	0.6	1.8
Horse Bean, young.....	10.0	26.2	4.6	17.2	23.7	5.3	5.2	2.0	11.3	5.3	24.3	0.6	1.9
	3.3	22.6	3.8	15.3	7.3	5.1	5.1	2.0	12.4	5.2	23.6	0.6	1.8
	.....	22.2	2.9	15.0	(+0.5)	4.9	5.1	1.7	15.0	4.8	21.1	(+0.2)	1.8
Horse Bean, old.....	30.0	29.8	5.7	19.5	41.9	5.5	5.6	2.2	13.5	5.7	24.5	2.4	1.9
	10.0	26.2	4.1	16.7	23.7	5.1	5.2	2.0	12.9	4.8	25.0	1.4	1.9
	3.3	19.6	2.9	16.3	-0.3	4.8	5.2	1.9	12.3	5.1	22.1	-0.4	1.9
Yellow Lupin, young.....	30.0	35.5	5.7	21.2	61.1	5.2	5.8	2.1	10.8	5.6	26.8	2.0	2.2
	10.0	30.5	3.8	16.5	10.9	4.8	5.4	2.1	13.8	5.1	25.6	2.3	1.9
	3.3	21.2	3.3	14.3	-0.3	4.8	5.1	2.0	8.3	5.4	23.1	-1.7	1.9
None.....	.....	20.9	2.9	15.8	(+1.8)	4.3	5.0	1.8	11.1	5.2	23.4	(-0.7)	1.9
	30.0	33.2	5.1	21.4	55.7	5.5	6.4	2.2	13.0	5.4	26.8	3.5	1.9
	10.0	25.9	4.0	15.9	43.9	5.1	5.6	2.1	12.1	5.0	23.9	0.8	1.9
Yellow Lupin, medium.....	3.3	20.8	3.7	15.5	2.9	5.5	4.6	2.1	12.1	5.0	23.4	0.2	1.9
	30.0	18.2	4.4	21.3	13.0	5.2	5.7	2.0	13.3	5.3	23.6	1.4	2.0
	10.0	18.9	3.4	20.2	9.4	5.2	5.2	2.2	13.6	5.3	22.4	0.8	2.0
Yellow Lupin, old.....	3.3	17.9	3.1	18.9	2.6	4.2	4.6	2.0	13.3	5.6	20.4	-0.5	2.2
	.....	20.1	3.5	15.3	.....	4.6	5.0	1.8	12.8	5.5	22.5	....	2.0
	Average checks.....	20.1	3.5	15.3	.....	4.6	5.0	1.8	12.8	5.5	22.5	....	2.0

TABLE 10  
*Dry weights on rich soil*  
Averages grams per pot

MANURES	gms.	1	1	2	3	4	5	6	2-6	7
		CORN	INCREASE	OATS	RYE	MILLET	BUCK- WHEAT	CORN	INCREASE	WHEAT
None.....	....	88.3	per cent (+9.2)	38	29	20	4.8	24.6	per cent (±0)	11.9
Sweet Clover, young.....	30.0	117.8	46.1	41	34	22	5.0	21.6	6.2	11.9
	10.0	106.1	31.5	37	31	20	5.3	22.5	4.6	....
	3.3	98.3	21.7	40	30	21	5.0	22.8	2.0	....
Sweet Clover, medium.....	30.0	108.3	34.2	40	34	23	5.1	21.3	6.1	....
	10.0	102.0	26.5	38	33	22	4.9	20.3	3.3	10.9
	3.3	93.3	15.5	39	33	21	5.1	21.3	2.6	10.9
None.....	....	80.5	(-0.5)	39	32	22	4.6	19.3	(+0.3)	11.5
Sweet Clover, old.....	30.0	107.5	33.3	43	35	21	5.1	19.3	6.1	....
	10.0	95.5	18.2	40	33	21	5.3	19.3	1.7	....
	3.3	91.3	13.0	39	35	21	5.3	19.0	2.4	....
Cowpea, young.....	10.0	104.3	29.2	37	35	21	5.5	18.8	0.7	12.0
	3.3	92.8	14.9	37	34	20	5.9	17.4	-2.0	11.9
Cowpea, medium.....	10.0	101.1	25.2	40	32	21	6.1	18.6	1.0	12.5
	3.3	90.5	12.0	40	33	22	5.6	17.6	1.5	....
None.....	....	79.6	(-1.6)	41	32	20	5.6	17.4	(+0.4)	....

Cowpea, old.....	30.0	105.6	30.9	40	37	24	5.5	18.0	6.3	....
	10.0	96.3	19.2	40	32	24	5.4	17.5	2.1	11.6
	3.3	88.5	9.5	39	33	22	6.0	15.3	1.0	11.5
Cowpea, ripe.....	30.0	101.4	25.6	45	35	22	6.1	20.6	10.7	12.4
	10.0	93.3	15.5	39	32	22	5.6	18.1	0.3	....
	3.3	88.4	9.4	40	31	22	6.3	17.1	0	....
None.....	....	72.1	(-11.0)	39	30	22	5.1	17.5	(+2.5)	....
	30.0	102.2	26.6	38	34	26	5.6	17.8	4.3	12.0
	10.0	95.1	17.7	42	30	24	5.4	18.9	3.4	12.1
Cowpea, frozen.....	3.3	90.2	11.7	40	31	23	6.0	18.9	2.1	11.0
	30.0	98.7	22.3	41	37	25	5.5	17.8	8.1	....
	10.0	96.7	19.7	38	31	22	5.4	20.1	0	....
Soybean, young.....	3.3	91.1	12.7	41	31	22	5.8	17.0	0.3	....
	....	82.8	(+2.4)	40	31	23	5.6	19.6	(+2.4)	12.1
	30.0	103.7	28.5	42	34	23	5.9	20.1	7.4	12.5
Soybean, medium.....	10.0	94.6	17.1	37	35	23	5.5	18.0	1.7	12.6
	3.3	88.0	8.9	39	36	21	5.1	18.8	3.0	....
	30.0	100.1	24.0	41	32	26	5.6	19.4	6.6	....
Soybean, old.....	10.0	94.3	16.7	39	31	25	5.4	19.3	2.8	....
	3.3	88.7	9.7	36	30	24	5.5	20.3	-0.6	13.0
	....	79.4	(-1.9)	41	28	22	5.3	17.1	-1.0	11.6
Average checks.....										
	....	80.9	....	40	30	22	5.2	19.3	...	11.8

TABLE 11

*Nitrogen in crops on poor soil*

Averages milligrams per pot

NITROGEN APPLIED	1	2	3	1-3	4	5	6	7	8	9	10	1-10
	CORN	CORN	OATS	RETURN per cent	COTTON	EYE	FLAX	MILLET	BUCK- WHEAT	CORN	WHEAT	RETURN per cent
None.....	113.3	45.2	98.6	.....	60.4	80.3	38.6	66.7	59.0	99.4	31.2	.....
Pea, medium.....	855.0	359.0	138.0	33.3	100.7	110.9	41.4	97.5	64.4	104.6	35.5	47.0
	285.0	212.5	117.0	36.5	82.6	91.1	37.1	82.7	48.5	103.4	.....	53.6
	94.1	153.6	102.5	32.7	83.6	78.3	33.3	70.4	51.8	100.2	.....	51.9
White Lupin, young.....	711.0	251.2	152.6	30.1	88.6	117.8	39.1	70.5	56.2	104.1	38.1	41.4
	237.0	161.2	123.8	28.7	96.1	84.7	37.4	78.4	52.0	100.6	.....	49.5
	78.2	134.5	116.6	45.5	85.9	89.1	31.0	61.4	55.9	94.3	.....	71.9
None.....	134.2	39.1	93.6	.....	83.4	78.8	34.8	51.7	58.9	92.3	30.2	.....
White Lupin, medium.....	534.0	196.7	135.4	23.8	105.6	84.8	37.2	73.3	66.1	97.6	36.1	35.8
	178.0	160.0	130.6	37.4	108.1	76.7	34.4	54.5	54.9	91.6	.....	54.1
	58.7	144.6	110.5	47.9	89.7	72.8	35.0	63.0	46.5	88.9	.....	71.4
White Lupin, old.....	489.0	189.1	136.3	24.4	112.9	83.5	35.1	58.7	67.1	94.6	32.8	34.7
	163.0	137.0	127.1	33.3	80.5	72.4	32.2	60.7	60.5	93.1	.....	38.1
	53.8	118.0	115.7	52.3	82.0	72.8	36.9	66.1	54.3	88.8	.....	71.9
None.....	122.2	45.2	106.5	.....	100.0	69.5	31.5	52.0	65.5	98.4	33.0	.....
Hairy Vetch, young.....	981.0	404.7	164.3	42.9	114.6	93.7	41.4	60.0	82.5	107.9	36.0	53.6
	327.0	255.6	128.5	56.4	102.4	77.1	35.5	51.0	69.3	103.4	.....	68.4
	107.9	151.5	123.8	62.4	105.1	86.0	35.3	50.5	66.0	95.4	.....	89.7

Hairy Vetch, medium.....	813.0	382.0	72.8	139.3	40.8	124.2	83.5	48.2	46.5	69.6	119.6	31.7	63.1
	271.0	191.0	49.3	146.4	45.9	109.5	82.4	41.8	63.5	72.1	110.0	28.0	72.3
	89.4	145.5	42.9	102.9	30.8	105.0	74.9	38.2	55.5	60.6	104.6	27.2	69.7
Horse Bean, young.....	297.0	188.7	52.9	120.4	34.1	121.9	74.9	38.8	64.4	62.0	104.5	28.1	53.8
	98.0	180.8	41.0	93.3	46.4	89.8	65.3	36.5	61.4	52.5	96.8	28.0	57.1
None.....	.....	139.8	32.5	91.5	.....	90.2	71.9	29.6	65.0	52.8	99.2	28.0	.....
Horse Bean, old.....	873.0	232.5	66.1	128.7	26.7	104.0	78.1	44.2	75.1	64.4	115.2	30.0	35.1
	291.0	175.5	47.2	116.9	26.2	102.0	72.3	36.8	74.4	54.2	105.0	30.0	41.4
	96.0	125.4	36.2	110.8	9.9	92.0	70.7	37.4	66.4	52.0	92.8	29.0	32.4
Yellow Lupin, young.....	900.0	312.4	69.0	148.4	29.8	90.0	82.4	38.4	68.4	59.9	120.6	33.2	33.9
	300.0	192.1	44.8	107.3	25.4	92.6	73.4	37.0	57.2	58.1	107.5	29.8	33.0
	99.0	123.0	40.0	94.4	0.5	92.6	72.5	44.9	41.5	60.5	108.6	29.8	33.0
None.....	.....	133.8	33.4	90.1	.....	80.0	70.7	29.9	61.1	63.4	107.6	29.5	.....
Yellow Lupin, medium.....	603.0	242.4	61.2	145.5	29.0	106.7	97.3	40.5	64.2	62.1	104.5	29.8	40.2
	201.0	168.4	46.4	106.5	25.8	101.5	89.0	48.9	61.2	53.5	105.2	28.9	54.6
	66.3	118.6	37.4	113.2	32.0	109.5	73.9	37.6	67.0	53.5	103.0	27.6	73.1
Yellow Lupin, old.....	381.0	121.9	50.6	153.4	15.1	110.8	86.6	40.6	52.0	63.1	94.4	30.0	29.7
	127.0	117.2	40.8	133.3	19.0	97.8	82.2	40.5	75.6	53.5	89.6	29.8	43.6
	41.9	100.2	40.9	134.2	9.7	89.9	72.0	37.8	72.8	57.1	89.8	33.4	50.0
Average checks.....	.....	128.7	39.1	96.1	.....	82.2	74.2	32.9	59.3	59.9	99.4	30.5	.....

TABLE 12

*Nitrogen in crops on rich soil*

Averages milligrams per pot

NITROGEN APPLIED	1	2	3	4	5	6	7	1-7
	CORN	OATS	EYE	MILLET	BUCK- WHEAT	CORN	WHEAT	RETURN per cent
None.....	593.5	209.0	203.0	106.0	86.3	206.7	178.5	.....
Sweet Clover, young.....	762.0	291.1	244.7	125.4	97.5	187.9	178.5	75.2
	254.0	218.3	229.3	126.0	97.6	209.1	.....	87.0
	83.8	220.0	201.0	123.9	93.0	203.0	.....	79.3
Sweet Clover, medium.....	804.0	208.0	227.9	151.1	101.6	200.2	.....	41.9
	268.0	207.0	231.0	129.8	93.0	186.6	176.6	79.2
	88.4	171.6	234.3	119.8	95.5	204.5	167.9	103.7
None.....	586.3	210.6	217.7	129.8	86.8	206.3	119.6	.....
Sweet Clover, old.....	693.0	245.1	248.5	123.9	91.8	216.2	.....	38.8
	213.0	204.0	221.0	113.4	104.5	179.2	.....	27.7
	70.3	226.2	231.0	111.4	101.3	182.3	.....	89.0
Cowpea, young.....	354.0	192.4	235.0	117.6	113.2	172.7	134.4	43.6
	116.8	222.0	241.0	104.0	108.5	198.4	133.3	102.5
Cowpea, medium.....	271.0	224.0	230.0	126.0	114.6	180.2	140.0	27.1
	89.4	192.0	228.0	132.0	103.5	193.6	.....	41.6
None.....	575.9	237.8	218.0	120.0	103.5	184.3	.....	.....

Cowpea, old.....	654.0	755.8	27.5	180.0	255.0	132.0	110.5	190.9	.....	38.1
	218.0	692.6	54.4	196.0	214.0	127.2	98.3	185.4	116.0	60.8
	71.9	597.7	51.9	206.7	247.0	118.8	118.8	174.4	119.0	127.7
Cowpea, ripe.....	600.0	709.9	24.8	207.7	252.0	138.6	111.6	214.1	136.4	42.3
	200.0	668.9	46.9	206.7	230.0	121.0	108.5	190.1	.....	70.1
	66.0	645.0	117.4	200.0	226.0	123.2	115.9	184.7	.....	171.6
None.....	.....	576.2	.....	179.4	216.0	103.4	92.8	183.8	.....	.....
Cowpea, frozen.....	591.0	775.0	35.2	186.2	228.0	145.4	101.8	199.3	112.8	44.0
	197.0	691.0	64.5	222.6	228.0	127.2	97.2	200.2	96.8	87.5
	65.0	697.5	149.4	192.0	217.0	126.5	121.8	194.8	124.3	206.9
Soybean, young.....	984.0	1178.8	62.1	172.2	259.0	109.9	108.3	185.1	.....	67.5
	328.0	876.8	96.4	205.2	223.0	121.0	104.8	194.9	.....	101.8
	108.2	677.4	114.5	207.7	223.0	121.0	108.4	192.1	.....	136.9
None.....	.....	567.7	.....	188.0	208.0	119.6	109.7	199.9	118.6	.....
Soybean, medium.....	696.0	785.6	88.9	277.2	228.0	138.0	103.7	192.9	150.0	45.1
	232.0	673.4	40.0	173.9	245.0	151.8	112.1	181.8	153.7	78.4
	76.6	670.3	102.8	187.1	252.0	100.9	100.6	180.4	.....	157.5
Soybean, old.....	645.0	730.0	25.4	217.3	221.0	111.8	113.6	221.1	.....	33.2
	215.0	646.8	27.7	179.2	217.0	122.4	105.3	216.1	.....	46.6
	71.0	636.5	79.4	215.0	207.0	103.2	112.6	196.8	184.0	181.2
None.....	.....	601.7	.....	221.4	196.0	110.0	106.0	174.3	148.5	.....
Average checks.....	.....	575.2	.....	207.7	216.7	114.8	97.5	192.6	141.3	.....



The percentage deviation of the total fresh weight of all manured crops from the average check is given in tables 2 and 3, together with the mean probable error calculated for all crops. A comparison of these figures with one another shows that the crop increases caused by the smallest application of green manures (equivalent to about 25 pounds nitrogen per acre) are in most cases not much larger, sometimes even smaller, than the single probable error. Undoubtedly the accuracy of these results is not very high, as is to be expected when such small quantities of organic nitrogen are applied.

Tables 7 to 12 show that the original productivity of the rich soil was about twice as high as that of the poor soil and that the total average production of all crops (including the buckwheat used as preliminary test crop) was on the unmanured check rows: on poor soil, 99.6 gm. dry weight with 810.8 mgm. N per pot; on rich soil, 211.9 gm. dry weight with 1638.1 mgm. N per pot.

TABLE 13  
*Average crop increases and nitrogen return on poor and on rich soil*

MANURE PER POT	DRY WEIGHTS, PER CENT INCREASE				NITROGEN, PER CENT RECOVERED			
	Poor soil		Rich soil		Poor soil		Rich soil	
	Crops 1-3	Crops 4-9	Crop 1	Crops 2-6	Crops 1-3	Crops 1-10	Crop 1	Crops 1-7
gm.								
30.0	51.0	3.1	30.2	6.9	29.4	41.5	35.0	47.5
10.0	26.7	1.1	21.5	2.0	33.5	54.8	46.8	64.5
3.3	5.7	0.3	12.6	1.4	33.6	61.2	73.9	127.1
Averages...	27.8	1.5	21.4	3.4	32.2	52.5	51.9	79.7

Accordingly, the crop increases caused by green manuring were larger and more lasting on the poor soil, especially with the heaviest application of legumes. The average percentage increase in dry weights and the average nitrogen return calculated from the data in tables 9 to 12 are shown in table 13.

On poor soil the first 3 crops were distinctly benefited by the green manuring, whereas very little effect was noticeable with the following 6 crops. On rich soil the first crop only was markedly influenced, and after 5 other crops were taken the effect had practically reached its end. The relations selected for the different quantities of green manures (9:3:1) are very clearly reflected in the crop increases on poor soil. Those for the total fresh weights are 7.5:3:1 and the relations of the dry weight increases in the fourth to ninth crop (3.1:1.1:0.3) are almost identical with those chosen for the green manures (30:10:3.3). On the rich soil the crop increases are disproportionately large with the smaller applications of green manures, obviously because of an enhanced mobilization of soil nitrogen, caused by more vigorous bacterial action in the manured pots.

The detailed figures contained in tables 11 and 12 show that with large applications of green manures the nitrogen returns are fairly similar with different plants and on different soils. With smaller applications, however, the rich

TABLE 14  
*Nitrification and nitrogen availability of green manures in poor and in rich soil*

GREEN MANURES		N NITRIFIED	N RECOVERED PER CROP
<i>Poor soil</i>			
Pea, medium.....		<i>per cent</i> 22	<i>per cent</i> 25
White Lupin {	Young.....	8	10
	Medium.....	14	14
	Old.....	7	6
Hairy Vetch {	Young.....	29	31
	Medium.....	25	28
Horse Bean {	Young.....	15	32
	Old.....	19	12
Yellow Lupin {	Young.....	8	13
	Medium.....	5	13
	Old.....	6	..
Averages.....		14	16
<i>Rich soil</i>			
Sweet Clover {	Young.....	19	52
	Medium.....	20	52
	Old.....	12	28
Cowpea {	Young.....	33	28
	Medium.....	19	2
	Old.....	8	45
	Ripe.....	5	65
	Frozen.....	3	80
Soybean {	Young.....	30	91
	Medium.....	23	57
	Old.....	16	44
Averages.....		17	50

soil is far superior; the nitrogen return rises in one case to more than 200 per cent. That the green manure nitrogen is more completely mineralized by the soil organisms, and that it is of comparatively greater effect upon the manured

plants when it is applied in moderate quantities, is indicated by the average percentage of nitrogen recovered on both soils after large, medium, and small applications, viz., 42, 55, and 61 per cent on the poor soil, 47, 65, and 127 per cent on the rich soil.

The results of nitrification tests presented in table 14 furnish additional information concerning the different behavior of the green manures in poor and in rich soil.

In poor soil, percentage nitrification and nitrogen return in the first crop agree very closely in most cases. Although in the nitrification tests approximately three times the maximum amount of green manure pots was used, the results obtained for the poor soil confirm the frequently recorded fact that nitrification and vegetation experiments may give similar results despite the different conditions under which they are made. With the rich soil, on the other hand, the nitrification tests gave results far below the nitrogen returns in the first crop. A comparison of the nitrification results for both soils, however, shows an almost identical behavior of the different materials, and a marked reduction in nitrate formation when more mature legumes were tested. The conclusion is justified, that the actual availability of green manure nitrogen was more accurately measured in the first crop by the nitrification experiments than by the vegetation tests.

Additional information on the increase in the humus decomposition in the rich soil, apparently caused by the accelerated microbial action in the soil lightly dressed with green manure, was secured from a special set of nitrification experiments in which the quantities of green manure applied agreed closely with those used in the pot tests; 0.3, 0.1, and 0.03 gm. dry substance were added to 100 gm. soil, respectively, with 12, 4, and 1.2 mgm. total nitrogen. Dried young vetch, an easily nitrifiable material, was added to the soil, and 6 tests were made in every case. The following confirmative results were obtained (per cent nitrogen nitrified): large quantity, 50.4; medium quantity; 68.3; small quantity, 195.1.

The stimulation of humus decomposition in the manured soils is shown likewise by the average nitrogen returns recorded graphically for the successive crops. (Fig. 2.) The main parts of the resulting curves reflect very clearly the gradually diminishing returns, but the initial deviations from these curves shown by the first crops leave no doubt that some other source of nitrogen must have caused this irregularity. That the humus nitrogen is responsible for this effect is further evidenced by the different behavior of the two soils. In contrast to the poor soil, the rich soil, because of its higher nitrogen content, shows no deviation from the curve to the negative side. The initial increase in the mineralization of soil nitrogen and its utilization by the first crop is followed by a compensating deviation from the curve to the negative side, indicating a temporary exhaustion of available soil nitrogen.

The gradual flattening of the availability curve in the third and fourth year after the green manures had been applied demonstrates very convincingly that in soils not exceptionally rich in humus nitrogen a 50 to 60 per cent

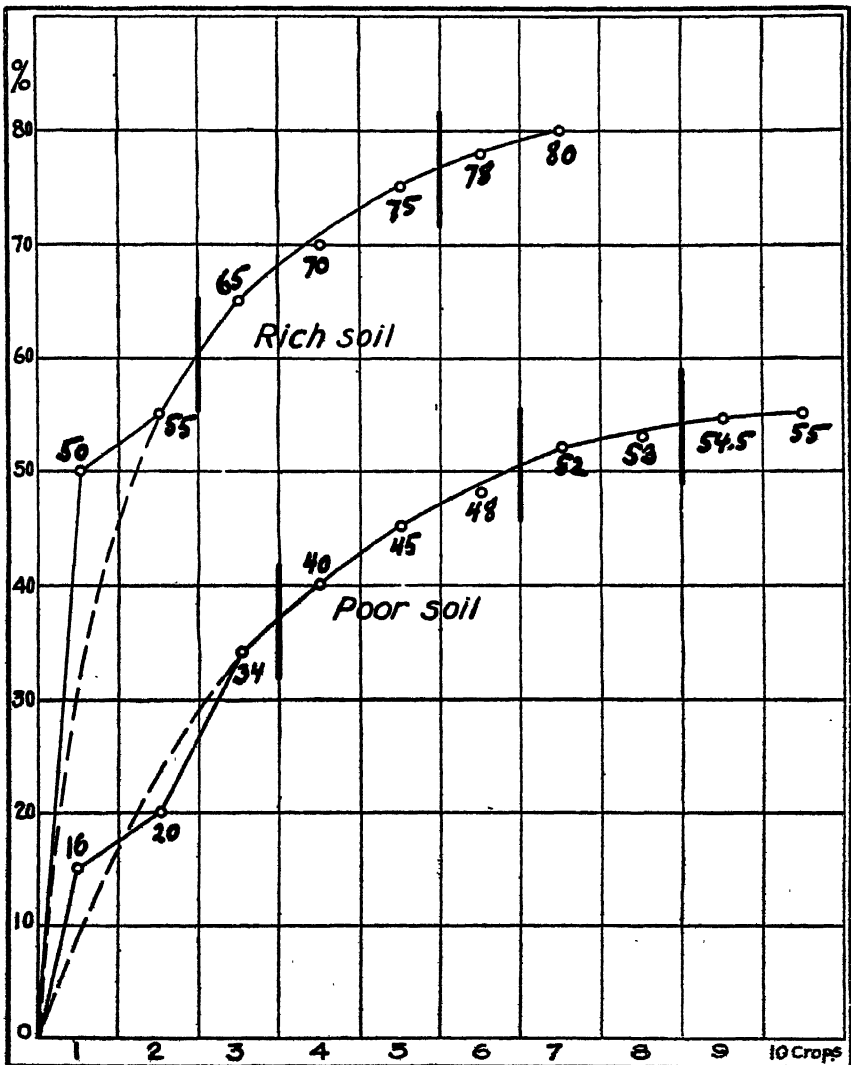


FIG. 2. AVERAGE NITROGEN RETURNS (PER CENT) IN 10 CROPS ON POOR SOIL AND IN 7 CROPS ON RICH SOIL

The short vertical lines separate the years in which the different crops were grown

nitrogen availability can be considered as an average return for green manuring. Soils poor in humus nitrogen naturally induce humus formation, and in them part of the organic nitrogen is firmly maintained in this form by microbial



1	24	23	19	42	..	3	24	..	1	6	..	36	66	39	53	3	52	117	149	115	103	79
2	2	13	4	2	..	16	19	16	..	..	..	16	..	24	14	..	..	..	..	..	..	..
3	7	26	8	..	..	36	5	36	..	26	8	..	..	20	21	12	40	14	1	6	50	..
4	2	14	25	7	10	..	13	..	10	16	18	13	6	..	..	19	6	13	18	6	..	..
5	4	11	1	..	..	..	..	..	..	..	..	..	..	..	10	7	30	28	37	10	5	22
6	..	2	6	4	5	7	4	7	12	7	12	14	14	..	5	1	..	..	3	..	..	6
7	12	..	..	2	7	13	6	13	..	12	12	..	3	..	..	..	..	..	..	..	..	76
8	..	1	1	..	..	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..
9	1	..	6	..	..	..	..	..	9	5	..	..	..	..	..	..	..	..	..	..	..	..
10	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Total...	52	90	70	57	32	72	71	72	33	72	50	79	105	89	103	42	128	172	208	137	158	183

3.3

action. Even after having been mineralized it may again be assimilated by fungi, algae, and bacteria.

However, more or less marked differences in the nitrogen availability become evident if the behavior of the various kinds of green manures is examined. Table 15 shows the percentage figures for the nitrogen returns in abridged form (without decimals). These figures exhibit clearly considerable differences among the green manures tested, and a summary of the initial and final effects (table 16) shows this even more definitely.

Pea and hairy vetch both gave a relatively large return in the first crop, but later the pea was outranked by the vetch. Horse bean acted differently according to its age: young material behaved much like pea and vetch, where-

TABLE 16  
*Percentage of nitrogen recovered in the first crop and in the following crops*

POOR SOIL	PEA	HAIRY VETCH		HORSE BEAN		WHITE LUPIN			YELLOW LUPIN		
	Medium	Young	Medium	Young	Old	Young	Medium	Old	Young	Medium	Old
Crop 1.....	25	31	28	32	12	10	14	6	13	13	..
Crops 2-10.....	26	39	40	24	24	44	39	42	20	46	41
Total.....	51	70	68	56	36	54	53	48	33	59	41
	SWEET CLOVER			COWPEA					SOYBEAN		
	Young	Medium	Old	Young	Medium	Old	Ripe	Frozen	Young	Medium	Old
Crop 1.....	52	52	28	28	2	45	65	80	91	57	44
Crops 2-7.....	28	23	24	46	33	31	30	33	11	37	44
Total.....	80	75	52	74	35	76	95	113	102	94	88

as old material was similar to white and yellow lupines, which always acted little on the first, but more on the following crops. Sweet clover and soybeans, like horse beans, showed a slower and less complete effect with increased age, whereas mature cowpeas gave higher returns, especially after exposure to frost.

The physical and chemical characters of old horse beans, lupines, sweet clover, and soybeans explain their reduced efficiency. Their cellulose content increases considerably and the nitrogen content is reduced; accordingly, they stimulate microbial assimilation of nitrogenous compounds and reduce the mineralization of organic substances. Cowpeas, on the other hand, remain, even when their seeds are mature comparatively rich in nitrogen and low in cellulose content. Their high efficiency is, therefore, not very sur-

prising. Nevertheless, why freezing especially, has further stimulated this growing nitrogen efficiency can not be fully explained at present, whereas in the nitrification test the results have decreased with increasing age in this as in other cases. (Table 14.) The causes of the exceptionally low returns shown by young and medium aged cowpeas are entirely problematical. The regularity of their appearance precludes their being caused by an experimental error, and the similar inferiority exhibited by young yellow lupines when compared with older material indicates that they are caused by some unknown physiological peculiarities. Unsatisfactory results recorded occasionally with cowpeas and with yellow lupines in field tests may have been the outcome of similar conditions.

#### DISCUSSION

The experiments described on the preceding pages have confirmed earlier findings insofar as an average nitrogen return of approximately 50 to 60 per cent of the applied green manure could be recorded. However, only one-half to three-fourths of this amount was contained in the first crop; the residual effect was distributed in a regularly decreasing manner during the next two or three years. The separate findings showed wider fluctuations than have been recorded before. In the first crop they varied from negative results up to an apparent nitrogen availability of 149 per cent, whereas the total effects ranged from 33 to 208 per cent. It was shown that these high returns are due to a stimulation of the mineralization of humus nitrogen under the influence of relatively small applications of green manure, as are often used in the field. It is possible, therefore, that green manuring may sometime lead to a depletion in soil nitrogen, a fact which never before seems to have been noticed.

The biochemical transformations proceed more slowly in the field, than in pot experiments so that this undesirable influence upon the humus nitrogen is less likely to play an important rôle. But it is difficult, if not impossible, to decide in most cases, how far data obtained in field experiments actually represent the nitrogen availability of the green manures used. As a rule, the plants are plowed under where they have been grown. Thereby the specific effect exerted by the growing legumes upon soil productivity is more or less obliterated by the processes started in the soil by the incorporated green matter.

This specific effect, however, must be known in order to decide correctly whether the application of green manure is physiologically and economically sound. At first glance, it seems beyond doubt that the turning under of a green manure crop containing 100 pounds nitrogen per acre with an availability of about 50 per cent represents an efficient and economic means of improving the soil. Nevertheless a thorough knowledge of the action of the growing legumes as such, may change this view more or less.



Since under suitable conditions the nitrification of green manure nitrogen proceeds fairly rapidly during the first few weeks, it is easily understood why little, if any, noticeable effect can be expected if green manure is plowed under in summer or in fall while the soil is still warm, and if the field is replanted several weeks or months later after heavy rains have washed out the nitrate which had been formed. Leguminous winter cover crops are generally much to be preferred, but in this case, too, it remains to be seen whether it is advisable to plow them under in spring or to use them in other ways.

#### SUMMARY

Field, greenhouse, and laboratory experiments upon the efficiency of green manures have furnished the following results:

(a) The nitrogen availability of green manures shows wide variations which are dependent on the quality and quantity of the green substances used and on the character of the manured soils. Small amounts of young materials, as a rule, give higher percentage returns than large quantities of old materials; but this rule is not without important exceptions. Cowpeas especially proved of greater value with increasing age, while young cowpeas, as well as young yellow lupins, showed an exceptionally low nitrogen availability. Frozen cowpeas displayed the highest efficiency.

(b) The average availability of green manure nitrogen was about 50 to 80 per cent if the green substances were added to the soil after they had been grown elsewhere and the tests were continued for several years. Similar results have been recorded repeatedly when green legumes were turned under where they had been grown. But it is an open question whether in such cases the effect is really the result of the green manuring, or whether it is more due to the influence of the growing legumes as such. The data recorded are in favor of the last named possibility.

(c) If green manures are incorporated into a soil not too poor in humus a general acceleration of the activities of the microorganisms living in the soil takes place with the result that the nitrification of the green manure nitrogen is accompanied by an intensified mineralization of the humus nitrogen. Accordingly, more nitrogen may be found in the first crop increases than has become available from the green manures. Occasionally on a rich soil more than 200 per cent of the nitrogen applied was returned within a few years. Nitrification tests made in the laboratory gave much lower figures than vegetation tests with this soil, whereas concordant results were obtained by both methods for a soil of low humus content.

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## WHY ARE SERPENTINE AND OTHER MAGNESIAN SOILS INFERTILE?

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General agreement exists among geologists and other students of soils respecting the marked inferiority as plant media, of soils derived from pure serpentine or from other highly magnesian rocks. No accord among scientists, has, however, been reached regarding the cause or causes of the infertility in question. The hypothesis which attempts to clarify the problem and to which many students of soils and plants have indicated their adherence, is briefly to the effect that the specific toxicity of magnesium accounts for the unsuitable nature of serpentine and similar soils as media for plant growth. Hilgard, among other investigators, accepted that hypothesis, particularly in view of Loew's well-known work on the rôle of calcium and magnesium in plant growth and on the lime-magnesia ratio. He added another view (2), however, to the effect that magnesian soils are usually deficient in "plant-foods" (4, 5).

It seemed to the authors that, inasmuch as the problem lends itself well to experimental test, it was high time to determine definitely the real answer to the question of the cause or causes of the infertility of serpentine soils. The following experiments, therefore, were planned and executed. Soils or freshly disintegrated serpentine rock was obtained from different locations in the hills of the Coast Range of the San Francisco Bay region, and submitted to the studies which are described below. The soils collected were as follows:

*No. 1.* From the west peak of Mount Tamalpais, Marin County. Disintegrating serpentine rock. No vegetation on it except an occasional tuft of moss on rock masses. Free from contamination with any other rock or soil.

*No. 2.* Vicinity of Mount Diablo, Contra Costa County. Taken from the top of a serpentine hill. No contaminating material. A very sparse grass vegetation existent.

*No. 3.* From same general location as No. 2, but at the foot of, and in a little vale between, two hills. Possibility of contaminating material. The soil was black, fine in texture, deep, and easily workable, thus distinctly different from the coarse material in No. 1 and No. 2. A fairly good grass vegetation existent, also scattered oaks and pine trees of fair vigor.

*No. 4.* From the side of a serpentine hill near Oakland, Alameda County. Disintegrated

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rock particles. No vegetation where sample was taken, but very sparse grass growth near place of sampling.

No. 5. Taken 3 feet from No. 4, among grass roots. The soil was only 6 inches deep.

No. 6. From a grassy knoll near No. 5. Only about one inch of soil and disintegrating rock. Grass very short and going to seed prematurely. Soil from among roots of grass.

No. 7. From among pine trees near No. 6. Soil about bare except for tree growth mentioned.

No. 8. From a location close to No. 4, 5, 6, and 7. Bottom of a knoll. Soil brown in color. A very poor growth of young eucalyptus trees.

No. 9. From a location near San Francisco. A black soil on disintegrated serpentine rock. Fairly good vegetation.

No. 10. From another spot in same location as No. 9. Disintegrated serpentine rock bearing a very poor vegetation.

#### COMPOSITION OF THE SOIL SOLUTION OF THE EXPERIMENTAL SOILS

Before proceeding to the determination of the cause of the well-known behavior of serpentine soils toward plants, it seemed highly desirable to learn something about the composition of their solution. To that end, a preliminary test was planned on a method to be employed for the purpose in subsequent work. The preliminary test consisted of obtaining the concentration

TABLE 1  
*Concentration of solution of Mount Tamalpais serpentine soil*

	C <sub>2</sub> H <sub>5</sub> OH DISPLACEMENT	ACETONE DISPLACEMENT	WATER EXTRACT 1:1
Total solids, <i>p. p. m.</i> .....	144	150	166
Freezing point depression, °C.....	0.021	0.021	0.026

of the solution of three samples of the Mount Tamalpais rock powder by the following methods:

(a) Parker's displacement method with acetone, (b) Parker's displacement method with alcohol, (c) water extract, using 1 part soil to 1 part water, and (d) freezing point depression of solutions obtained by the three foregoing methods, in addition to the gravimetric determinations upon them.

Table 1 gives the results obtained on the three soil samples in question. Only an average of the values obtained is given, since agreement between the samples was very good.

Even the foregoing simple preliminary tests gave us a significant hint of the answer to our problem in the remarkably low concentration of the soil solution, but it seemed at once desirable to have more detailed data on the composition of these soil solutions. Inasmuch as there were no significant differences between the values given in table 1 as representing different methods, the water extract method was used in the subsequent determinations. In addition to analyzing the soil extracts thus obtained, however,

conductivity determinations were made on the same solutions, and the pH was determined throughout. Briefly, the following procedure was employed in the analyses of the ten samples of soil described above. The soil was passed through a 1-mm. sieve. It was then shaken with an equal weight of distilled water for one hour and a half in a mechanical shaker. The extract was filtered through a Pasteur-Chamberland filter and analyzed by the standard methods in vogue in modern laboratories. The nitrate content of the extract was determined on two soils by two methods so as to check on the reliability of the data. The methods used were the phenol-disulfonic acid method and the Devarda method. The two methods checked to within one part per million. The conductivity of the soil extracts was determined by the standard methods. The pH values were determined electrometrically,

TABLE 2  
*Analysis of serpentine soils*  
(In parts per million)

SOIL NUMBER	pH VALUE	TOTAL NITROGEN	NO <sub>3</sub>	CONDUCTIVITY SPECIFIC RESISTANCE	TOTAL SOLIDS	NON- VOLATILE SOLIDS	Ca	Mg	HCO <sub>3</sub>	PO <sub>4</sub>
				<i>ohms</i>						
1	8.20	?	Trace	..	166	92	20	21	150	Trace
2	8.12	140	13	3,336	324	157	20	22	20	4
3	8.12	115	8	4,200	247	145	14	31	18	..
4	8.18	78	2	2,291	..	..	16	20	21	..
5	8.01	112	2	2,957	..	..	..	..	23	..
6	5.98	286	6	1,888	..	..	..	..	7	..
7	7.90	50	2	4,308	250	117	..	..	16	..
8	8.10	133	3	3,052	358	208	11	22	19	..
9	7.65	136	2	2,176	450	210	19	45	18	..
10	8.11	96	2	2,232	..	..	..	..	12	..

using 10 gm. of soil and 25 cc. of CO<sub>2</sub>-free water for every soil. The results of all these analytical studies are set forth in table 2.

The results of the analyses as submitted in table 2 are interesting even at a casual glance. In the first place, they check the freezing point determinations in testifying to the strikingly low concentration of solutes in the material in question. In the second place, the data for pH determinations show with only one exception that the soils or rock powders are decidedly alkaline. In the third place, the conductivity measurements stated in ohms as specific resistance check the freezing point determinations as well as the analyses. Finally, the lack of PO<sub>4</sub> ion in all solutions but one testifies to the effectiveness of the alkalinity in removing phosphorus from the soil solution. The values for Ca and Mg are not striking except to indicate that the magnesium content cannot well be the dominant factor in determining the sterility of

the soil in question. It is, perhaps, worthy of special comment that all the soil and rock powders studied are shown in table 2 to possess very small quantities of nitrate. Further comment with respect to this aspect of our problem will be made below.

Although the quantity of volatile matter in the soils is not high, it should be remarked that when the serpentine materials were dried at 100°C., they suffered losses of 30 to 40 per cent in weight and were difficult to bring to constant weight. This indicates a probable very high content of combined water in the molecules making up the rock material.

#### PHYSIOLOGICAL EXPERIMENTS

In view of the interesting analytical data briefly discussed above, the authors decided upon a program of experimentation in cultures which might be calculated to determine the validity or invalidity of the conclusions suggesting themselves from table 2, which are as follows:

1. Magnesium is probably not responsible for the sterility of serpentine soils.
2. The high pH of the soil solutions in question is a suspicious factor in the problem.
3. The very low content of important ions in the solutions, and particularly the  $\text{NO}_3$  and  $\text{PO}_4$  ions, constitute another suspicious factor in the problem.

In the culture experiments, barley was grown in extracts of some of the soils in question, and in artificially compounded solutions of similar composition. The serpentine soils at first chosen for making extracts were the Mount Tamaplais and the Mount Diablo soils since both gave chemical characteristics strikingly similar, as evidenced in table 2, and yet were strikingly dissimilar in appearance, as the descriptions given above will readily make clear.

The barley plants were grown, therefore, in culture solutions placed in fruit jars of approximately one liter capacity. For each kind of solution, jars were used in quadruplicate, and 5 plants were grown in every jar, thus 20 plants for each type of medium allowed some control of individual variation among the plants. To serve as a control culture solution for comparison with the soil extracts and with other similar solutions, a medium composed of 0.004 *M*  $\text{MgSO}_4$ , 0.004 *M*  $\text{Ca}(\text{NO}_3)_2$ , and 0.004 *M*  $\text{KH}_2\text{PO}_4$  and with about 0.5 atmosphere osmotic pressure, was employed. In cases in which two salt constituents were added in the test solutions, the total quantity used was such as to be equal to that of the foregoing control solution, namely, 0.012 mol of salts per liter. In other cases in which but one salt was added, a quantity of the salt equal to 0.005 mol per liter was employed in order to keep the individual ionic concentrations approximately comparable with those of the control solution. In the natural soil extracts, of course, the concentrations of solutes were lower than in the artificially prepared or modified solutions, as exemplified in the Mount Tamaplais soil, which has a concentration equivalent to less than 0.005 mol per liter. The solutions were not changed during

the culture period, but distilled water was added from time to time to make up for losses by transpiration, and  $\text{FeSO}_4$  was added as needed.

For a clearer presentation of the data on the culture experiments, the soils tested will be considered separately.

*The Mount Tamalpais soil*

Since it was difficult to obtain large quantities of this soil for preparation of soil extracts, only three series of culture solutions were prepared therewith. The other eight series were made up artificially to be identical with the first three. The kinds and amounts of salts necessary for the artificially prepared solutions were determined by calculating the reaction values of the salts of the original solution from which the molal concentration may be calculated.

TABLE 3  
*Analysis of the Mount Tamalpais soil*

IONS	QUANTITY FOUND	EQUIVALENT WEIGHT	REACTION VALUE	CONCENTRATION
	<i>p.p.m.</i>			<i>mols.</i>
Na.....	12.0	23.0	0.52	0.00052
K.....	6.4	39.0	0.17	0.00017
Mg.....	21.0	24.3	1.72	0.00172
Ca.....	20.8	40.7	1.02	0.00102
			3.43	0.00343
$\text{CO}_3$ .....	13.0	76.3	0.34	0.00034
$\text{SO}_4$ .....	5.4	96.0	0.11	0.00011
Cl.....	15.0	35.5	42.0	0.00042
$\text{HCO}_3$ .....	153.0	61.0	2.52	0.00252
			3.39	0.00339

The reaction value in each case is found by dividing the quantity in parts per million found by the equivalent weight. The method is made clear in table 3.

The plants in the Mount Tamalpais series were grown for a little over five weeks in the fall of the year. The results obtained are recorded in table 4.

Because of an accident, the plants in the Mount Tamalpais series were lost before their dry weights could be determined. Table 4 shows, however, the lengths of tops and roots obtained in the several cultures. It is clear from the data in table 4 that two factors limit the growth of plants in extracts from the Mount Tamalpais soil. One is the high pH and the other is the lack of  $\text{NO}_3$  and  $\text{PO}_4$  ions. In this case, there is a smaller effect resulting from the correction of the pH than from the addition of necessary ions. Nevertheless the correction of the pH is clearly effective when the  $\text{NO}_3$  and  $\text{PO}_4$  ions are not limiting factors, as a comparison of series IV with series II indicates. These data would seem to give tangible support for the conclusions drawn from the analytical data submitted in table 3.





TABLE 5  
*Data on growing barley in the aqueous extract of the Mount Diablo (soil No. 2) and in similar solutions*

DESCRIPTION	COMPOSITION OF SERIES									
	No. I	No. IA	No. II	No. III	No. IV	No. V	No. VI	No. VII	No. VIII	No. IX
	Control 0.5 atm.	Control 0.5 atm.	Soil extract	Soil extract + KNO <sub>3</sub>	Soil extract + NaNO <sub>2</sub>	Soil extract + HCl	Soil extract + HCl + KNO <sub>3</sub>	Soil extract + NaNO <sub>2</sub> + HCl	Soil extract + KNO <sub>3</sub> + NaH <sub>2</sub> PO <sub>4</sub>	Soil extract + KNO <sub>3</sub> + NaH <sub>2</sub> PO <sub>4</sub>
pH value before growing.....	7.2	5.5	8.1	8.1	8.1	5.5	5.5	5.5	5.5	8.1
Length of tops, cm.....	65	64	25	42	40	24	48	38	78	45
Length of roots, cm.....	25	25	75	45	28	40	35	55	30	28
Average weight of tops, gm.....	4.86	3.72	0.53	1.67	1.44	0.61	2.98	1.95	6.39	2.25
Average weight of roots, gm.....	1.14	1.29	0.24	0.81	0.40	0.30	1.10	0.50	1.52	0.58
Inflorescences.....	30	30	1-3	7	10-20	1-3	18	12	30	18
Average of total weight, gm.....	4.5	5.06	0.79	2.6	1.88	0.91	3.83	2.33	7.77	2.50

*The Mount Diablo soil*

At this juncture, it seemed essential, however, to obtain more extensive and complete data with other serpentine soils. An experiment similar to the one just described, therefore, was started with extracts of one of the Mount Diablo soils (No. 2) instead of the Mount Tamalpais soil. The results are given in tables 5 and 6.

The data in tables 5 and 6 not only confirm, but considerably strengthen those of table 4. The additional evidence furnished in the values for the dry weight of the plants and in the number of heads produced gives conclusive proof that the magnesium content of the serpentine soil extract is not the cause of the depression of plant growth therein. On the other hand, they confirm

TABLE 6  
*Weight of tops and roots of barley plants grown in the solutions given in table 5*

JAR NUMBER	DESCRIPTION	SERIES									
		No. I	No. IA	No. II	No. III	No. IV	No. V	No. VI	No. VII	No. VIII	No. IX
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	Tops	4.93	3.80	0.54	1.67	1.47	0.64	2.98	1.82	6.33	2.08
	Roots	1.07	1.43	0.23	0.80	0.41	0.30	1.00	0.47	1.33	0.50
2	Tops	4.79	3.60	0.49	1.72	1.48	0.57	2.90	1.94	5.87	2.80
	Roots	1.00	1.21	0.25	0.79	0.40	0.26	1.03	0.51	1.46	0.68
3	Tops	4.80	3.72	0.58	1.62	1.36	0.64	3.03	2.13	6.63	2.03
	Roots	1.20	1.18	0.26	0.78	0.42	0.30	1.24	0.52	1.65	0.60
4	Tops	4.91	3.80	0.52	1.67	1.52	0.58	3.02	1.92	6.77	2.10
	Roots	1.30	1.35	0.23	0.85	0.38	0.35	1.13	0.50	1.65	0.53
Average of total weight....		4.50	5.06	0.79	2.60	1.88	0.91	3.83	2.33	7.77	2.50

the conclusion, which may be drawn from the length of tops and roots yielded in the cultures, that the high pH of the solutions and the deficiency first in  $\text{NO}_3$  ion and secondly in  $\text{PO}_4$  ion, limit the growth of plants in those solutions. This evidence is the more striking since the Mount Diablo soil is totally different in appearance and age from the Mount Tamalpais soil which it resembles so strongly in its physiological effects. The more subtle factors common to serpentine soils and not their outward appearances are, therefore, their dominant features from the physiological standpoint.

In order to strengthen the evidence, the results of an experiment with still another Mount Diablo soil (No. 3) conducted as a parallel with soil No. 2 are given in tables 7 and 8.

Again, there is evidence in a soil of very different appearance from either of the foregoing, of the existence of a common and dominant set of factors

determining their physiological value. (Tables 7 and 8.) Again, we have striking evidence of the effectiveness of the factors common to serpentine

TABLE 7

*Data on growing barley in the aqueous extract of the Mount Diablo soil (soil No. 3) and in similar solutions*

DESCRIPTION	COMPOSITION OF SERIES								
	No. XI	No. XII	No. XIII	No. XIV	No. XV	No. XVI	No. XVII	No. XVIII	No. XIX
	Soil extract	Soil extract + $\text{KNO}_3$	Soil extract + $\text{NaNO}_3$	Soil extract + $\text{HCl}$	Soil extract + $\text{KNO}_3$	Soil extract + $\text{NaNO}_3$	Soil extract + $\text{KNO}_3$ + $\text{NaH}_2\text{PO}_4$	Prepared solution 0.003 mol. (page 298)	Soil extract + $\text{KNO}_3$ + $\text{Na}_2\text{HPO}_4$
pH value before growing	8.2	8.2	8.2	5.5	5.5	5.5	5.5	7.3	8.2
Length of tops, cm. ....	30	38	34	28	42	40	82	29	70
Length of roots, cm. ....	90	60	62	75	60	56	27	34	27
Average weight of tops, gm. ....	0.79	1.50	1.28	0.72	2.15	1.83	7.37	0.83	4.28
Average weight of roots, gm. ....	0.32	0.68	0.35	0.30	0.85	0.42	1.64	0.27	1.22
Average of total weight, gm. ....	1.10	2.15	1.62	1.04	2.95	2.34	9.01	1.08	5.50
Inflorescence. ....	1-3	6	5	3	8-10	6	30	3-5	30

TABLE 8

*Weight of tops and roots of barley plants grown in the solution given in table 5*

JAR NUMBER	DESCRIPTION	SERIES								
		No. XI	No. XII	No. XIII	No. XIV	No. XV	No. XVI	No. XVII	No. XVIII	No. XIX
		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	Tops	0.73	1.67	1.44	0.80	2.10	1.82	7.29	0.84	4.41
	Roots	0.29	0.68	0.36	0.30	7.50	0.40	1.70	0.20	1.34
2	Tops	0.78	1.38	1.17	0.77	2.23	1.84	7.43	0.82	4.32
	Roots	0.32	0.57	0.30	0.27	0.78	0.42	1.80	0.28	1.29
3	Tops	0.74	1.60	1.37	0.62	2.26	1.77	7.21	0.86	4.27
	Roots	0.26	0.72	0.42	0.30	0.98	0.40	1.50	0.23	1.13
4	Tops	0.92	1.33	1.13	0.75	2.00	1.87	7.48	0.82	4.12
	Roots	0.42	0.64	0.31	0.35	0.89	0.45	1.56	0.28	1.10
Average of total weight. ....		1.10	2.15	1.63	1.04	2.95	2.35	9.00	1.08	5.50

soils and again, there appears to be no evidence that the magnesium content of the soil extracts is the toxic, inhibiting, or dominant factor.

In view of the decisive nature of the foregoing results, it seemed unnecessary to test the solutions of the other serpentine soils which had been collected as above described.

#### GENERAL DISCUSSION

There is a striking agreement between the general effects of the serpentine soil solutions, regardless of the apparent differences between the soils as regards their effects on the growth of barley. The minor differences in their effects as noted in the tables are easily explained on the basis of the different periods of the year in which the several experiments were conducted and on the different periods for which the cultures were grown. This would seem to indicate that the effects of the solutions in question on barley plants are not explicable on any irregular or accidental constituents of serpentine soils, but on constant and dominant characters.

Our data show, further, that there is confirmation in the conditions found (*a*) in the native vegetation on serpentine soils, (*b*) in the kind of plants grown on soil extracts from them and on artificial solutions made to simulate them in composition, and (*c*) in the analytical data showing the outstanding characters of the serpentine soils.

The small amount of magnesium found in all the serpentine soil extracts when viewed in the light of the results obtained by Gericke who grew plants successfully in solutions whose salt constituents were seven-eighths magnesium salts, would, in itself, give no ground for attributing the partial or complete sterility of serpentine soils to that factor. But when it is realized, as the foregoing experiments compel one to do, that the addition of a lacking ion or a change in the pH, or both, in a serpentine soil extract with the same magnesium content, changes it from a poor to a good medium for a barley plant, a full answer is given to the question which forms the subject of this paper. In fact the authors have even added magnesium to some of their culture solutions in the foregoing experiments without rendering them toxic or more toxic thereby.

It is not the magnesium content but the high pH value which is the dominant and consistent characteristic of serpentine soil extracts. It is known, of course, that the weathering of serpentine rock results in a mixture of carbonates and silicates. These must give rise to the  $\text{HCO}_3$  and  $\text{SiO}_3$  ions found on analysis in the serpentine soil extracts which, in turn, on hydrolysis give the high pH values above shown. At equilibrium between the serpentine soil and its solution, the pH value is about 8.1. In growing on such a soil, a plant would tend to lower the pH, but this would result in further hydrolysis of the compounds in question with resultant maintenance of a high pH. It is only in this very indirect sense that Mg salts in serpentine soils can be said to be responsible for their infertility.

The very dilute solution which characterizes serpentine soils is worthy of special comment. The authors have found a very small colloid content in

all the serpentine soils. This may, perhaps, account in some measure for the lack, of retentiveness of important ions for plants by the soils in question, but it is more likely that the unique chemical character of the rocks contributing to such soils is a more important factor in accounting therefor. These considerations would seem to offer the key to the solution of the problem of rendering serpentine soils more fertile.

#### SUMMARY AND CONCLUSIONS

1. A study was made of 10 widely different serpentine soils from different localities.

2. All of the soils were analyzed and the conductivity of their solutions was determined. It was found that:

All soils were poor in available important ions.

The nitrate content was low even where the total nitrogen was considerable.

Most of the soils had a pH value generally of about 8.1.

Although serpentine soil is derived from a magnesian rock, the ratio of magnesium to the total concentration of the soil extract was not necessarily high.

A few of the soil extracts were analyzed for phosphate and potassium, and were found to be deficient in these constituents.

3. Three of the soils were studied by growing barley in culture solutions (prepared either by adding certain constituents to their soil extracts, or to solutions like their soil extracts, but artificially prepared.) It was found that:

The addition of nitrate improved the growth of the plants.

The lowering of the pH value improved the plant growth where necessary ions had been added.

The addition of potassium improved the root development of the plants.

The addition of phosphate increased the growth of the plants.

The addition of magnesia to soil solutions from one soil had no effect on the growth of the barley.

The growth of the plants was at its maximum when several essential ions were added and the pH value was lowered.

4. The experiments with barley also showed, like the analytical data, that the soil extracts were poor in nitrate, phosphate, and potassium, chiefly the first two.

5. There is a strong parallelism between the composition of serpentine soils and the vegetation found on them as well as the behavior of barley grown in extracts from them.

6. The infertility of serpentine soils is not caused by a too high content of soluble magnesium, but by a high pH and a deficiency in certain ions, chiefly nitrate and phosphate. This conclusion reverses all the teaching on this subject heretofore given, especially that as regards the toxicity of magnesium in serpentine soils.

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# THE TOLERANCE OF PLANTS FOR NaCl

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A number of circumstances, economic and scientific, have conspired to invest the subject of salt effects in plant growth with outstanding importance in the realm of plant physiology. When we remember that the root medium in nature is a weak salt solution whose changing composition within a season is an important determinant of plant growth, that the introduction of salts into that medium may exert marked influences on the absorptive capacities of the plant for necessary and unnecessary ions, that large concentrations of sodium salts and others may depress and even exterminate plant growth, that the resistance of plants to such baneful effects of excessive quantities of salts may lead to important physiological and genetic discoveries—we have before us a few of the many cogent reasons for studying systematically and intensively the relations of salts to plant growth.

Such were the considerations which induced the authors to undertake to contribute another chapter, in addition to those already contributed by one of the authors, to the story of salt relations of plants. The aim in these experiments was to determine the tolerance of plants for NaCl under extreme conditions, to note if any stimulating effects ever enter, and to discover if different kinds of plants react differently to the same concentration of the salt.

## PLAN OF EXPERIMENT

In formulating the procedure to be adopted in their experiments, the authors decided that all soil culture experiments on the subject of the toxicity of NaCl or of the tolerance of plants for NaCl are seriously at fault, in that uncontrollable factors or at least unknown factors are introduced into the experiment by the use of soils as media. Both culture solution experiments and sand culture experiments are on record in sufficient number to show that soil and possibly other solid media do not lend themselves well to the prosecution of research on the tolerance of plants for salts. In the case of soils particularly, the well-known phenomenon of the replacement of bases which follows upon the heels of the introduction of any salt like sodium chloride into the soil, is likely to bring about a condition which masks the real nature of the effects of a salt upon a plant. At this juncture in the development of the science of plant physiology and its associated sciences, the phenomena just



referred to, including the replacement of bases and the antagonism between ions, are so thoroughly appreciated by plant physiologists as to require no reference to the long list of articles which testify to the interest they have stimulated.

In view of what has been said, we determined to employ the culture solution method in the present studies. Although, to be sure, the constituent salts used in the culture solution do exert some effect upon the salt whose specific relations with the plant are under investigation, such effects cannot well be considered as being of great magnitude inasmuch as the sodium chloride under study in our work is used almost throughout in such large concentrations as to overshadow in its effects any neutralizing reactions which other salts present in the medium with it may exert thereon. The basic culture solution which was used in these experiments had the following composition in grams per liter, the water of crystallization being included:

Ca(NO <sub>3</sub> ) <sub>2</sub> .....	1.721
KNO <sub>3</sub> .....	0.321
KH <sub>2</sub> PO <sub>4</sub> .....	0.249
MgSO <sub>4</sub> .....	0.993

The ionic composition of this solution in parts per million was as follows:

Ca.....	292
NO <sub>3</sub> .....	1100
Mg.....	98
PO <sub>4</sub> .....	174
SO <sub>4</sub> .....	372
K.....	194

To this solution there was added throughout, four drops of a 5 per cent solution of ferrous sulfate to each 2-liter jar. Similar amounts of iron were added whenever the condition of the plants indicated a need. In each 2-quart Mason jar, 1700 cc. of the nutrient solution just described was placed. After having introduced into the jar a sufficient amount of concentrated NaCl solution to make up the desired concentration of NaCl, the total amount of the solution was brought up to 1900 cc. in every jar. The plants were set into the paraffine cork stoppers after the usual fashion employed in such experiments. Every concentration of salt was tested in quadruplicate. In the case of wheat and barley, five plants were set in each jar; in the case of peas, only three plants. All the plants were grown in a greenhouse where the temperature was not controlled but varied within a considerable range.

#### RESULTS OF THE WHEAT SERIES

The concentrations of NaCl employed in the wheat series varied from 500 p.p.m. to 15,000 p.p.m. with intervals of 500 p.p.m. between each of two successive sets of jars. The wheat seedlings were set out in December, 1920. In the second week of their growth in the jars, it was possible to detect dif-

ferences in the heights of the tops of the plants growing in concentrations of NaCl above 8500 p.p.m. In the concentrations below that, the tops seemed to be of uniform height throughout. No growth, as judged by increase in length, was detected in concentrations of NaCl above 14,000 p.p.m., but at this time all the plants were still alive. As regards the root development during the second week, it was possible to notice two maxima in root length. The roots in the control set of jars which received no sodium chloride were normal, those growing in 500 p.p.m. were only about three-fourths the length of the former; and those at higher concentrations of NaCl were showing a gradual lengthening over those in the 500 p.p.m. concentration until a concentration of 2500 p.p.m. was reached, where the roots appeared to be as long as they were in the control jars. Beyond 4000 p.p.m. there was a gradual decrease in the length of the roots until a concentration of 14,000 p.p.m. was reached, beyond which there was no growth evident. The roots even at this early stage were beginning to disintegrate, as shown by a slimy coating on their surfaces.

Four weeks after planting, the tops of the plants at concentrations of 6500 p.p.m. and above were noticeably thinner and a little shorter than at any concentration below. Even at this date there was little difference in height among the plants in all the sets up to 6500 parts NaCl per million. The tops of the plants all appeared to be healthy even in the highest concentration of 15,000 p.p.m. where no growth was taking place. Nevertheless, the gradual disintegration of the plants in the high concentrations of sodium chloride was visible at this point. In the root systems at the same date, the two maxima for root length and development which were noted above had become accentuated so that the roots in a concentration of 500 parts NaCl per million were no more than one-half as long as those in the control. Those in 1000 p.p.m. were about the same length as those in 500 p.p.m. Then, as the concentrations of NaCl increased, the length of the roots increased gradually up to 3000 parts NaCl per million, where the roots were slightly longer than those of the controls. From 4000 p.p.m. NaCl to higher concentrations there was a gradual decrease in the root length, the roots becoming more and more stubby, and slightly brown and discolored above 8000 p.p.m. NaCl.

At the third observation, about two weeks subsequent to the one just described, the color of the plants was good in all the sets except those of 15,000 p.p.m. NaCl. A few jars in which higher concentrations of NaCl than 15,000 p.p.m. were employed contained no living plants, and only a few plants were alive in concentrations above 14,000. In the lower concentrations, the vigor of the plants was clearly manifest. The plants tillered well and there seemed to be little difference between the control plants and those growing in concentrations of NaCl up to 4,000 p.p.m. Beyond the latter concentration the plants gradually decreased in thickness of stalk and width of leaf. Nevertheless, the plants growing in concentrations of 10,000 p.p.m. NaCl appeared normal when viewed alone. The roots appeared to be normal, and only a comparison of these plants with the control showed the latter to be superior.

The roots at this observation were long, white, and branched in the control cultures. The roots in 500 p.p.m. NaCl were markedly dwarfed and showed a tendency to send out long, thick, unbranched roots from the crown. This was true in all the four jars of this same concentration. Again it was noticed, as on the previous occasion, that the root system gradually increased in length with the increase of concentration of NaCl up to 4,000 p.p.m., where they were equal in length to those of the control. There was a slight difference between these and the control roots in other respects, however, in that they

TABLE 1  
*Tolerance of wheat plants for NaCl*  
Dry weight per culture (5 plants)

CONCENTRATION NaCl	TOPS						ROOTS	
	1	2	3	4	Total	Average	Total	Average
p.p.m.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
000	11.8	11.8	11.6	9.3	44.5	11.1	6.9	1.70
500	13.8	14.8	13.8	16.6	59.0	14.7	9.8	2.45
1,000	14.1	12.3	14.9	14.9	56.2	14.1	8.6	2.15
1,500	18.8	15.4	15.5	16.1	65.8	16.4	10.1	2.50
2,000	16.1	15.7	16.4	15.9	64.1	16.0	10.3	2.56
2,500	18.0	13.0	17.5	14.9	63.4	15.8	9.6	2.40
3,000	15.7	18.7	21.6	17.3	73.3	18.4	11.2	2.80
3,500	16.0	17.5	16.5	17.9	67.9	17.0	9.6	2.40
4,000	12.4	17.4	21.0	17.7	68.5	17.1	10.5	2.60
4,500	16.3	17.9	17.0	18.1	69.3	17.3	10.6	2.65
5,000	20.6	21.0	18.4	19.6	79.6	19.9	12.2	3.00
5,500	14.7	11.0	15.8	14.3	55.8	14.0	9.1	2.30
6,000	19.9	18.3	21.6	9.7	59.5	14.9	9.0	2.30
6,500	17.5	12.1	11.1	18.2	58.9	14.7	9.8	2.50
7,000	7.2	14.8	15.3	18.9	56.2	14.1	8.5	2.10
7,500	17.0	16.0	11.5	13.3	57.8	14.4	9.5	2.40
8,000	5.1	10.2	11.8	13.3	40.4	10.1	6.1	1.50
8,500	7.0	6.7	8.2	10.2	32.1	8.0	5.1	1.30
9,000	6.3	6.0	6.2	6.9	25.4	6.3	4.6	1.20
9,500	8.4	4.5	4.0	1.9	18.8	4.7	5.4	1.40
10,000	5.9	4.3	0.6	1.9	12.8	2.5	2.2	0.55

were slightly thicker, the branches were shorter, and the color slightly browner. Beyond 4000 p.p.m. concentration up to 14,000 p.p.m., there was a steady falling off in length in the root systems. In concentrations between 7000 and 12,000 p.p.m. NaCl the root branches were short, allowing the roots to be readily separated from one another.

Two weeks following the third series of observations (8 weeks after planting), the tops and the roots of the plants both showed differences in length over those noted earlier. There seemed to be at this point a gradual increase in height of the tops as one examined the concentrations from 500 to 4500 p.p.m.

NaCl. The control plants were still better than those in 500 p.p.m. NaCl. The maximum height of the tops was at a concentration of 4500 p.p.m. at this point in the growth of the plants. Beyond 13,000 p.p.m., all plants were dead. In the case of the roots, the differences in length which were so marked two weeks previously were at this date not so great, the tendency being seemingly in the direction of an equalization in the length of the roots in all the series up to 4500 p.p.m.

The last observations were made when the plants were about nine weeks old and only a few days before harvesting. The differences in length of root which are described above had almost entirely disappeared. Nevertheless, the roots of the plants in concentrations of 500 p.p.m. NaCl were still the shortest of the whole series except those in which distinct injury had occurred, namely in concentrations of 7500 p.p.m. or above.

The experiment was terminated about March 10, 1921. The plants were all dried and weighed. The roots were separated from the tops and, in order that the data might be subjected to statistical study, the top of each plant was weighed separately. Tables 1 and 2 give the data obtained on the dry weights of the plants which were harvested as follows: table 1 gives the results of the total weights of tops and roots from each jar, and table 2 gives the weight of each plant top separately. It is perfectly clear from these data that when tested by the solution culture method, NaCl does not become toxic to the wheat plant when grown under the conditions described until a concentration of approximately 8000 p.p.m. is reached, and at all concentrations of NaCl below 8000 p.p.m., the growth of the tops and roots, and particularly the former, is markedly stimulated by NaCl. When the yields of dry matter of individual plants or of groups of five plants together in every jar are compared with those of the control cultures in which no NaCl was included, but which were otherwise entirely suitable for the growth of the wheat, as the yields in the control cultures indicate, it is apparent that these are not accidental occurrences. It is also apparent that at 8500 p.p.m. and above, NaCl becomes very toxic and depresses the growth of the wheat plant until at 10,000 p.p.m., only about one-fifth of the dry weight of the control cultures is obtained. The break in the curve on dry weight yields is a fairly sharp one beyond the concentrations of 7500 p.p.m. NaCl. The dry weights of the roots parallel fairly closely those of the tops, so far as the important breaking point in the curve is concerned, at any rate. It is evident from these results that the lethal concentration of NaCl for the wheat plant under the conditions in question is somewhere in the vicinity of 10,500 p.p.m. when plant growth for the whole normal life period is considered. It is probably much higher than that for a very short period of say from two to four weeks of the life of a plant, as the observations above indicate.

All this is very different from the usual conception of the relation of common salt to the growth of the wheat plant. The fact that NaCl, under certain conditions, will markedly stimulate the growth of the wheat plant at all

TABLE 2

*Tolerance of wheat plants for NaCl*

Dry weight of individual tops

CONCENTRATION NaCl	JAR 1						JAR 2						JAR 3						JAR 4				MEAN DRY WEIGHT TOPS	DRY WEIGHT OF ROOTS				AVERAGE DRY WEIGHT OF ROOTS				
	g.m.		g.m.		g.m.		g.m.		g.m.		g.m.		g.m.		g.m.		g.m.		g.m.		g.m.			g.m.		g.m.			g.m.		g.m.	
00002.6	2.0	1.9	1.7	3.6	2.9	1.7	2.4	2.5	2.3	3.0	2.2	2.4	1.7	2.3	2.0	2.7	1.3	1.6	1.7	2.23	±	0.082	0.1	6.1	8.1	5	1.7					
50002.2	3.6	3.2	2.3	2.5	2.2	3.4	3.1	3.4	2.7	2.6	2.9	2.5	2.9	2.9	5.3	3.4	2.1	3.3	2.5	2.96	±	0.102	6.2	4.2	0.2	8	2.5					
1,0002.4	3.2	3.1	2.7	2.7	2.5	1.4	3.8	1.5	3.1	2.5	2.2	2.9	2.9	4.4	3.7	2.7	2.9	2.6	3.0	2.81	±	0.102	5.2	5.2	2.1	4	2.2					
1,5003.6	4.2	2.4	3.0	5.6	3.1	2.8	2.5	3.8	3.3	3.2	2.4	2.7	4.0	3.2	3.6	2.1	4.0	2.8	3.6	3.29	±	0.122	4.2	5.2	4.2	8	2.5					
2,0002.7	4.4	3.4	2.6	3.0	2.9	3.3	3.3	3.5	2.9	4.0	4.3	2.5	2.6	3.0	3.1	4.1	2.7	3.5	2.5	3.27	±	0.092	2.2	7.2	7.2	7	2.6					
2,5003.4	3.1	4.4	3.4	3.7	1.2	1.6	3.3	3.7	3.2	2.2	3.8	4.7	3.2	3.6	2.2	2.8	2.6	3.5	3.8	3.17	±	0.132	8.1	5.2	8.2	5	2.4					
3,0003.3	3.3	3.7	3.7	1.7	3.5	5.2	2.9	3.4	3.7	5.8	4.6	3.3	3.7	4.2	3.5	2.7	4.0	3.6	3.5	3.66	±	0.132	4.2	7.3	3.2	8	2.8					
3,5003.4	2.6	3.9	2.4	3.7	2.6	3.4	3.4	3.9	3.2	3.8	3.4	3.1	3.1	3.1	3.8	3.9	2.5	2.6	5.1	3.40	±	0.092	1.2	2.2	5.2	8	2.4					
4,0002.0	1.7	3.2	3.1	2.4	3.6	2.8	3.9	2.8	4.3	4.3	3.7	3.7	4.5	4.8	4.1	3.4	2.6	4.1	3.5	3.43	±	0.122	0.3	0.2	5.3	0	2.6					
4,5004.1	2.7	3.4	2.9	3.2	2.7	3.3	4.3	3.6	4.0	2.2	4.3	3.5	3.5	3.5	3.6	4.4	2.3	3.5	4.3	3.47	±	0.102	3.2	7.3	1.2	5	2.7					
5,0002.7	3.0	4.1	5.0	5.8	3.7	6.0	3.5	3.3	4.5	5.0	3.1	4.0	3.4	2.9	3.9	4.7	4.1	4.6	2.3	3.98	±	0.153	4.3	3.2	5.3	0	3.0					
5,5003.0	2.1	2.7	3.7	3.2	2.4	2.4	2.9	1.7	1.6	2.1	3.0	3.4	3.3	4.0	3.3	2.1	3.5	2.4	3.0	2.79	±	0.092	3.1	6.2	8.2	4	2.3					
6,0002.1	1.4	1.8	2.2	2.4	2.2	5.1	4.0	3.3	3.7	3.8	6.1	4.1	4.4	3.2	2.2	2.8	0.8	1.6	2.3	2.98	±	0.181	7.3	0.2	6.1	7	2.3					
6,5003.6	3.0	3.2	3.1	4.6	3.3	2.7	1.5	3.0	1.6	2.4	3.2	2.4	1.1	2.0	3.5	3.5	3.3	3.3	4.6	2.95	±	0.133	0.2	0.2	4.2	4	2.5					
7,0001.5	1.0	1.5	1.9	1.3	3.3	3.6	2.5	3.1	2.3	4.0	2.0	3.8	2.9	2.6	5.2	3.1	3.7	3.1	3.8	2.81	±	0.151	5.2	4.2	0.2	6	2.0					
7,5003.2	5.0	2.2	4.0	2.6	3.0	3.4	3.2	3.9	2.5	2.7	2.1	2.2	2.7	1.8	2.5	2.3	4.1	2.0	2.4	2.89	±	0.122	5.2	4.2	3.2	3	2.4					
8,0001.0	1.5	0.6	1.0	1.0	2.1	1.8	2.4	1.4	2.5	1.9	2.6	2.4	2.9	2.0	2.5	2.5	2.5	2.7	3.1	2.02	±	0.101	1.1	7.2	0.1	3	1.5					
8,5002.6	1.0	0.9	1.5	1.0	1.6	1.2	1.9	1.1	1.9	1.5	2.0	2.0	1.7	1.0	1.2	1.1	2.5	2.7	2.7	1.60	±	0.090	9.1	1.1	6.1	5	1.3					
9,0001.7	1.6	1.6	1.4	0.0	2.0	1.5	0.6	1.9	0.0	1.3	1.2	1.0	1.0	1.7	1.8	1.6	1.4	0.5	1.6	1.40	±	0.061	0.1	0.1	5.1	0	1.2					
9,5001.9	1.2	2.0	1.3	2.0	1.0	0.6	0.9	0.0	0.0	1.5	0.5	0.75	0.9	0.3	..	..	..	..	..	1.20	±	0.081	4.0	7.0	8.1	5	1.4					
10,0000.8	1.8	1.2	1.6	0.6	0.9	0.9	1.1	0.7	0.7	..	..	..	..	0.6*	..	..	..	..	..	1.00	±	0.071	2.0	2.0	2.0	6	0.6					

\* Total top weight.

concentrations from 500 to 7500 p.p.m. is a very striking fact in itself. But the extreme resistance of the wheat plant to the effects of NaCl is even more striking and the behavior of the plants in their earlier stages of growth, showing an initial depression in root and top development at the lower concentrations of 500 and 1000 p.p.m. NaCl and a stimulation beyond those points, is perhaps the most striking fact of all and one which is extremely difficult to explain on the basis of our present knowledge.

#### ANALYSES OF PLANT ASH IN THE WHEAT SERIES

Evidence has been adduced repeatedly in support of the more or less close relationship which exists between the absorption of ions by roots and the quality and quantity of plant growth attained. Knowledge on this point, however, is at the present time fragmentary and does not allow formulation of any quantitative idea with respect to this relationship. Although the task is an extremely laborious one, the authors deemed it worth while to carry out analyses of the ash of plant tops in the foregoing wheat series. The results of these analyses are set forth in table 3. The irregularity of the data given testifies to the errors to which analytical procedures in this regard are subject. There are, nevertheless, some interesting lessons, or at least interesting indications, attaching to the analytical data in question.

The plant seems to possess a mechanism for regulating its total ash content within certain fairly narrow limits, until a high concentration of NaCl is reached, when unusually large absorptions of salts occur which are reflected in the total ash contents of the plants. It is interesting to note that the marked increase in the ash content of these plants, which makes a pronounced deviation from a fairly uniform ash content in all the other series, is found in the plants grown in 8000 p.p.m. NaCl. That is the very point where a marked depression in the yield of roots and tops is evident. When the individual constituents of the ash are considered, there are some which require little or no comment. For example, the  $\text{PO}_4$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{Al}_2\text{O}_3$ , and  $\text{SiO}_2$  content is reasonably uniform throughout and is seemingly slightly or not at all affected by the changes in the NaCl content of the solutions in which the plants were grown. This is also true apparently for the potassium, though the analytical errors for this element are larger and the variability seems to be slightly greater as a result. In the case of the other ions, however, there are some very striking changes, as between their content in the ash of plants grown without NaCl and in plants grown with varying quantities of NaCl. Probably the most striking of all these is the case of the calcium ion. It will be noticed in a study of the data on calcium of the plants in the different series that even at 500 p.p.m. NaCl, there is a sharp depression over the control in the amount of calcium in the ash. The plants grown in 500 to 1500 p.p.m. NaCl have only one-third to one-half as much calcium as the plants in the controls which received no NaCl. The ash of plants grown in higher concentrations of NaCl than those named contained even less calcium, which, with one or two excep-

tional figures probably representing analytical errors. is fairly uniform throughout all of the concentrations of NaCl in question. It is certain that there is only approximately one-fourth as much calcium in the ash of plants grown in concentrations of 2.000 p.p.m. NaCl and above as in the ash of the control plants. Whether this depression in the calcium content is beneficial to the growing cells of the wheat remains to be answered by further study. There are some other lines of evidence which seem to converge on the point that such a benefit may be conferred on the wheat plant by reducing its calcium

TABLE 3

*Absorption of ions from solutions containing NaCl as shown by ash analysis of wheat plant tissue*

CONCENTRATION NaCl	PERCENTAGES, DRY WEIGHT											
	Total ash	SiO <sub>2</sub>	Al <sub>2</sub> O <sub>3</sub>	Fe <sub>2</sub> O <sub>3</sub>	Ca	Mg	Cl	SO <sub>4</sub>	PO <sub>4</sub>	Na	K	N
<i>p.p.m.</i>												
000	7.91	0.15	0.09	0.04	1.11	0.20	0.12	2.31	1.12	0.09	1.73	1.97
500	6.09	0.07	0.06	0.02	0.41	0.21	0.63	1.00	1.23	0.67	1.50	1.15
1,000	6.62	0.09	0.07	0.03	0.42	0.01	0.94	0.95	1.17	0.32	1.75	*
1,500	8.95	0.14	0.07	0.03	0.51	0.20	1.45	1.19	1.11	1.17	1.70	1.16
2,000	7.46	0.14	0.06	0.03	0.37	0.18	1.46	0.92	1.10	1.16	1.44	1.49
2,500	7.15	0.18	0.05	0.04	0.31	0.01	1.44	0.67	1.07	1.24	1.36	*
3,000	6.98	0.09	0.05	0.02	0.26	0.13	1.55	0.69	1.03	1.45	1.50	1.33
3,500	7.80	0.10	0.06	0.02	0.30	0.13	1.80	0.86	1.13	1.30	1.56	1.05
4,000	7.23	0.11	0.06	0.02	0.20	0.09	1.86	0.66	1.02	1.17	1.62	1.33
4,500	8.00	0.22	0.09	0.03	0.25	....	1.79	0.72	0.97	1.50	1.38	1.21
5,000	7.13	0.10	0.09	0.02	0.62	....	1.64	0.60	0.87	1.27	1.59	1.12
5,500	7.43	0.09	0.05	0.02	0.21	0.10	1.92	0.56	1.08	1.12	1.64	1.24
6,000	8.35	0.11	0.06	0.02	0.28	0.12	2.71	0.50	0.94	1.41	1.86	1.29
6,500	6.97	0.09	0.10	0.02	0.14	0.08	1.79	0.04	0.99	1.00	1.14	1.03
7,000	6.93	0.10	0.08	0.02	0.15	0.12	1.88	0.51	0.95	1.19	1.47	1.01
7,500	8.89	0.17	0.06	0.03	0.22	0.01	2.65	0.54	1.00	1.15	1.24	1.15
8,000	10.42	0.09	0.15	0.03	0.24	0.14	3.73	0.47	1.19	2.25	1.71	1.56
8,500	10.11	0.11	0.07	0.02	0.27	0.14	3.62	0.68	1.09	2.14	1.53	1.61
9,000	11.70	0.12	0.06	0.02	0.26	0.11	4.66	0.69	1.38	2.27	1.83	....
9,500	12.65	0.08	0.17	0.02	0.32	0.04	5.14	0.70	1.12	2.62	1.81	....

\* Lost.

content. In regard to the magnesium we find a similar situation as that which obtains in the case of the calcium, but the depression is, perhaps, not quite so marked as in the former case. It is noteworthy, however, that in the ash of some of the plants receiving NaCl little or no magnesium is found. This, too, may have a greater physiological significance than we are able, in the light of our present knowledge, to discern. So far as the chloride and sodium ions are concerned, only brief comment is necessary. Obviously with the increase of NaCl in solution an increase in the sodium and chloride of the plant ash is expected. It is noteworthy, however, that over a large range of concentrations of NaCl there is a fairly constant (analytical errors being taken into ac-

count) amount of chloride in the ash of the plants. Here again as in the case of the total ash we find a sharp and rapid rise in chloride content above 7000 p.p.m. NaCl in the culture solution. The same is true for the sodium ion, where the rise is much more sharp and begins at 8000 p.p.m. NaCl. It is interesting to note besides that, as several other recent investigations on absorptions of ions have indicated, the sodium and chloride ions evidently do not enter the plant or reach the tops at the same rates. Moreover, the absorption of chloride ions is noticeably faster at the lower concentrations of NaCl than at the higher concentrations of NaCl. To what extent this phenomenon is related to the permeability of the cells of the root and the top, is an open question. Not the least interesting of all the ions, is the sulfate ion. It will be noted that the introduction of 500 p.p.m. NaCl into the control solutions depresses the amount of the sulfate ion in the ash to a very marked degree. This depression lasts to the same extent up to a concentration of 2000 p.p.m. NaCl, when there is a further marked depression which remains fairly constant for all of the higher concentrations of NaCl. Apparently the chloride ion in some way substitutes itself for the sulfate ion or prevents the latter's entrance at the normal rate. All in all, it would seem that studies on the ash constituents of plants grown in such solutions are, in spite of the labor involved, well worth while, especially if the analytical methods, can be so much farther refined as to reduce the limits of error appreciably.

For a somewhat different reason than the other data, we have also included in table 3, figures on the total nitrogen of the plants grown in all the series. It is perfectly clear that NaCl in all concentrations reduces the percentage of nitrogen in the wheat plant to a marked extent. Even the smallest amount of NaCl used, namely 500 p.p.m., depressed the nitrogen content of the dry matter by approximately 40 per cent. This is probably indicative of the interference by the chloride ion with the penetration of the nitrate ion just as it interferes apparently with the penetration into the cell of the sulfate ion. It is evident from the figures on the nitrogen content of the dry matter in these plants that the total yield and appearance of a plant, as has been shown before, may give no indication of the nitrogen content thereof. A limited nitrogen supply at the disposal of the root systems would seem to be adequate for making maximum growths of plants if other conditions are satisfactory.

#### RESULTS OF THE BARLEY SERIES

The experiments with barley were carried out in the winter and spring of 1921-22. The details of the experiment, which are somewhat different from those of the wheat series, will be described separately.

The nutrient solution employed was the same for the barley series as for the wheat series but the NaCl was added to the several cultures in concentrations which did not comply, as regards intervals between successive concentrations, with the system employed in the wheat series. Each treatment was supplied in sextuplicate instead of quadruplicate as in the wheat series. This allowed



of 30 plants for every treatment and gave a better basis for statistical treatment of the data obtained at harvest. Table 4 gives the data with regard to the concentration of the NaCl used in the different cultures. An accident, through which most of the plants in one of the barley series were burned, made necessary the running of another series in the spring of 1922. The results on the plants saved from the first barley series, which was run in the fall of 1921, are also given in tables 6 and 7, because of some interesting facts which they bring out. Moreover, the water lost by transpiration was determined throughout the barley series, because the authors thought it would be interesting to determine how the salt content of the solutions influenced water losses under the conditions obtaining in this experiment. The methods used

TABLE 4  
*Tolerance of barley plants for NaCl*  
Dry weights, water transpired, and derived functions

NaCl	DRY WEIGHT TOPS PER PLANT	P.E. MEAN	DRY WEIGHT ROOTS PER PLANT	TOTAL DRY WEIGHT PER PLANT	H <sub>2</sub> O TRANSPIRED BY 30 PLANTS	RATIO OF H <sub>2</sub> O TRANSPIRED, TO DRY WEIGHT
	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>	<i>liters</i>	
0	3.30	±0.08	0.48	3.78	68.8	610
500	3.82	±0.09	0.46	4.28	70.7	560
1,000	3.45	±0.11	0.41	3.86	66.3	550
1,500	3.58	±0.11	0.40	3.91	61.9	530
2,000	4.02	±0.10	0.65	4.47	62.0	480
3,000	3.98	±0.14	0.41	4.39	52.2	420
4,000	3.66	±0.13	0.42	4.08	44.0	390
5,000	3.56	±0.14	0.42	3.98	41.6	370
6,000	3.66	±0.13	0.34	4.00	33.2	310
7,000	3.13	±0.19	0.24	3.37	21.8	320
9,000	0.71		0.72	0.83	7.3	310
11,000	0.46		....	....	3.2	260
13,000	0.20		....	....	1.1	...
15,000	0.11		....	....	....	...

in germinating the seeds and in setting out the plants were the same as those used in the wheat series. The solutions were changed once during the growing period. The second series of barley plants having been set out on January 26 and harvested on April 11, the change of solution took place on March 20. It was observed from the beginning that barley plants may continue to live for some time in high concentrations of NaCl without making any evident growth. In fact, the leaves of the plants may be dead and the roots look unhealthy, and yet the plants may be transferred to solutions free from NaCl and they will begin to make growth and continue to grow normally.

Seven days after placing the plants in the solutions, those in concentrations of 18,000 p.p.m. NaCl or in excess thereof had turned yellow and were making no growth. Plants in concentrations of 21,000 p.p.m. NaCl finally died with-

out making any growth. Plants in concentrations of 15,000 p.p.m. NaCl and below showed growth by actual elongation of the stem and the formation of new leaves. The difference between the behavior of the plants in concentrations of 15,000 to 18,000 p.p.m. NaCl is quite distinct. Table 5 indicates the number of plants which died in the higher concentrations and the time after planting at which such deaths took place. The death of many of these plants occurred only after the plant had made considerable growth. This emphasizes again the point which has been made in other papers issued from this laboratory, that as nearly as possible the full growing period of the plant must be allowed in such experiments. Table 6 indicates similar results

TABLE 5  
*Tolerance of barley plants for NaCl*

The number of plants in the various concentrations dying before completion of experiment\*

TIME FROM PLANTING	CONCENTRATION OF SOLUTION IN P.P.M. NaCl						
	6,000	7,000	9,000	11,000	13,000	15,000	18,000
<i>days</i>							
10—							
15—							
20—			1	1	2	4	7
25—							
30—		1			1		21
35—				4	7	6	
40—							
45—	1		1	2	3	3	
50—		1	3		4	1	1
55—							
60—							
65—		3	5	10	3	7	
70—							
75—			12	4	9	5	
80—							
Total...	1	5	22	21	29	26	29

\* Thirty seedlings originally in each solution.

obtained with the plants in the first barley series. At the time of harvesting, all the plants in concentrations of 9,000 p.p.m. NaCl and below were generally in a healthy condition. It will be seen from a study of table 4, in which the dry weights of the tops and roots of the individual plants are given, that the dry weight of barley plants was greater in all the concentrations of NaCl from 500 to 6,000 p.p.m. inclusive, than in the control series which received no NaCl. The appearances of the plants were in accord with the dry weights, inasmuch as the heights of all the plants receiving NaCl up to concentrations of 6000 p.p.m. were greater than the heights of the plants receiving no NaCl. It will also be noted in table 4 that there is a very definite and marked de-

pression in the water requirement of the barley plants after the NaCl content of the culture solution is increased. As a coincidence, it is noticed that here, as in the wheat series, the concentration of 8000 p.p.m. NaCl seems to be the critical one where the marked depression in plant growth occurs.

The two maxima for roots and tops which were noted in the case of the wheat series are not apparent in the barley series, and the stimulating effect of NaCl to barley plants under the conditions of the experiment is not nearly so marked as in the case of the wheat series. The roots were much more de-

TABLE 6  
*Tolerance of barley plants for NaCl*

The number of plants in the various concentrations dying before completion of experiment\*

	CONCENTRATION OF SOLUTION IN P.P.M. NaCl															
TIME FROM PLANTING	7,500	8,000	8,500	9,000	9,500	10,000	10,500	11,000	11,500	12,000	12,500	13,000	13,500	14,000	14,500	15,000
days																
10—																
15—																
20—																
25—																
30—																
35—						1					2			1		
40—																
45—																
50—																
55—				3	1	5	6	9	6	11	8	5	8	8	9	9
60—																
65—																
70—				1	5		8	5	10	14	14	15	15	13	13	10
75—																
80—																
85—																
90—	2	3	13	11	16	8	3	2			5		7	7	7	10
Total..	2	3	13	15	22	14	17	16	16	25	29	20	30	29	29	29

\* Thirty seedlings originally in each solution.

pressed in their development at concentrations beyond 5000 p.p.m. NaCl than were the tops, the differences between the 5000 and 6000 p.p.m. concentrations being very marked. In concentrations of 13,000 p.p.m. NaCl and above, the roots made practically no increase in length and were markedly discolored. In consonance with the results obtained by Muenscher (1) and others, the authors' data show that there is no parallelism between the amount of water transpired and the dry weights of barley plants produced. The curve shown in figure 1 obtained for the dry weights produced and the water transpired (curve B), indicates that there is a continuous fall in the curve

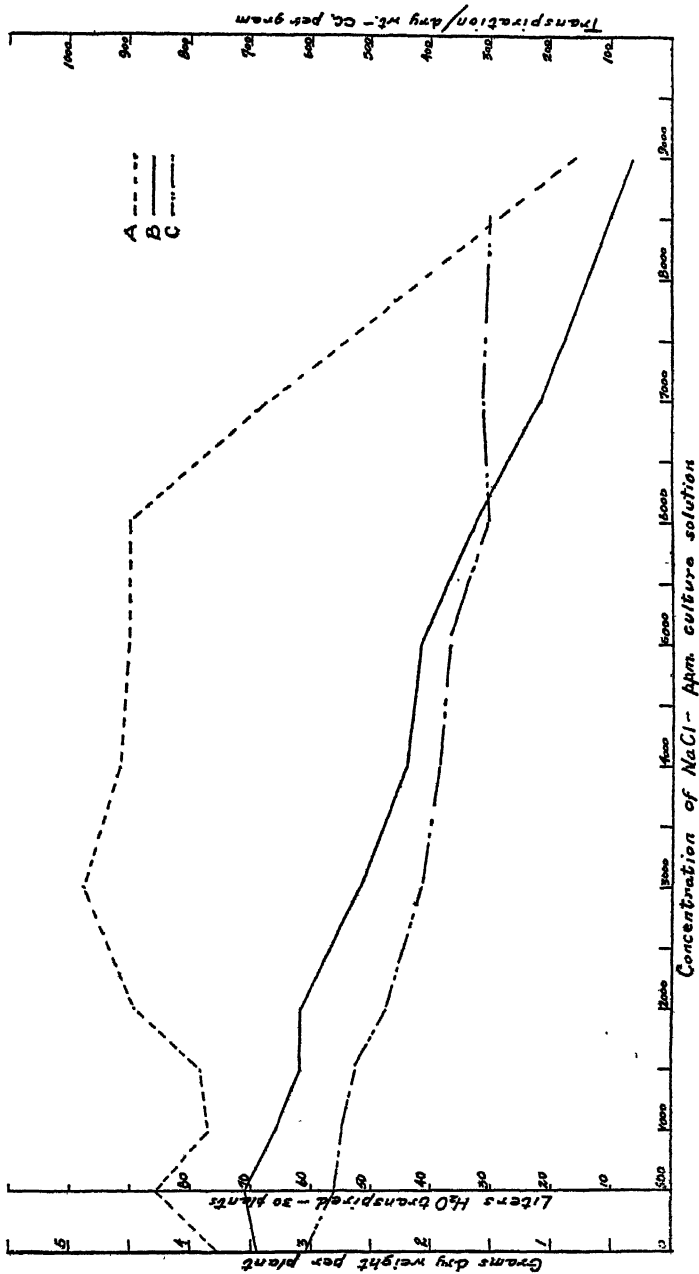


FIG. 1. THE EFFECT OF VARIOUS CONCENTRATIONS OF NaCl ON THE GROWTH OF BARLEY  
 A = total dry weight; B = total water transpired; C = ratio of water transpired to total dry weight

for water transpiration but an appreciable rise in the curve for the dry weight (curve A), and a fall when a sufficiently high concentration of NaCl is reached. This same figure gives an interesting comparison between the transpiration coefficient and the concentration of the medium (curve C). It will be seen that until concentrations of over 6000 p.p.m. NaCl are reached, the points fall very close to a line which is practically straight. A comment will be made later on the probable cause of the difference in the response between wheat plants and barley plants to the several concentrations of NaCl.

We have referred above to some interesting results which were obtained from the first series of barley with those plants in the series which were not burnt. Table 7 gives the results in question. It will be noted in that table that 500 p.p.m. NaCl proved to be markedly stimulating to the barley plants but that no other concentration of NaCl among those recorded in table 7 gave stimulation to barley growth over that obtained in the control. In fact, in the solutions containing 2000 p.p.m. NaCl, there seems to be a slight depres-

TABLE 7  
*Tolerance of barley plants for NaCl*  
Dry weights, water transpired, and derived functions—first experiment

NaCl	DRY WEIGHT TOPS PER JAR	P.E. MEAN	DRY WEIGHT ROOTS PER JAR	P.E. MEAN	TOTAL DRY WEIGHTS PER JAR	RATIO TOP ROOTS	P.E. MEAN
<i>p.p.m.</i>	<i>gm.</i>		<i>gm.</i>		<i>gm.</i>		
0	7.94	±0.28	0.89	±0.049	8.85	9.0	±0.37
500	12.57	±0.69	2.16	±0.140	14.73	5.7	±0.17
1,000	7.38	±0.05	1.06	±0.057	8.44	6.9	±0.17
1,500	7.59	±0.27	0.87	±0.062	8.46	8.6	±0.50
2,000	6.36	±0.05	0.99	±0.120	7.35	7.0	

sion in the growth of barley. It is interesting to compare these results with those obtained in the second barley series which are given in detail above. It seems obvious that the climatic complex under which plants are grown in such experimental work is of great moment in determining the effect of other environmental factors on plant growth. The very marked stimulation to barley growth exerted by 500 p.p.m. NaCl in this case is very striking. It is noteworthy, besides, that the ratio of tops to roots in regard to the dry matter produced is much higher in the control than it is in the cultures which received NaCl, and peculiarly enough, where the stimulation of the barley plants is obtained, the ratio was the lowest. The reason for this is that the roots in the cultures containing 500 p.p.m. NaCl had been stimulated so much more than the tops as to lower the ratio. The marked difference in the degree of stimulation of roots and tops in the cultures receiving 500 p.p.m. NaCl held throughout the growing period of the plants and was noticeable a few days after the plants were set out.

## RESULTS OF THE PEA SERIES

Only three seedlings per jar were set out in the case of the pea series. Otherwise, the same culture solution was used as in the other series. The concentrations of NaCl employed and the data obtained are given in table 8. The experiment was started January 28, 1922, and the plants were harvested on March 28. Dwarf garden peas were used. The weather was rather cold during the early part of the growing period of these plants but conditions improved in the latter part of the growth period. Nodules were noted on the plants in only a few jars in the large series which was arranged in this experiment. The development of the nodules did not seem to have any effect on the reaction of the plants to the NaCl. Flowers were observed on February

TABLE 8  
*Tolerance of pea plants for NaCl*  
Dry weights, water transpired, and derived functions

NaCl*	DRY WEIGHT TOPS PER JAR	P.E. MEAN	DRY WEIGHT ROOTS PER JAR	TOTAL DRY WEIGHTS PER JAR	WATER TRANSPIRED, 6 JARS	RATIO WATER TRANSPIRED, TO DRY WEIGHT
<i>p.p.m.</i>	<i>gm.</i>		<i>gm.</i>	<i>gm.</i>	<i>liters</i>	
0	6.48 ±0.18		0.91	7.39	16.1	370
500	6.84 ±0.16		0.96	7.80	16.9	360
1,000	6.88 ±0.34		0.99	7.87	16.6	360
1,500	7.32 ±0.23		0.91	8.23	15.6	320
2,000	6.18 ±0.17		0.94	7.12	14.1	330
3,000	6.24 ±0.06		1.07	7.31	12.4	310
4,000	5.78 ±0.23		0.84	6.62	10.3	260
5,000	4.46 ±0.20		0.86	5.32	8.5	270
6,000	2.89 ±0.23		0.65	3.54	6.2	230
7,000	1.92 ±0.20		0.47	2.39	2.9	230
9,000	0.73 ±0.03		0.01	0.74	0.8	...

\* In addition to the concentrations of NaCl here noted, the following were employed: 11,000, 13,000, 15,000, 18,000, and 20,000. For observations and remarks, see test.

23 and when the plants were harvested, many of them bore well-filled pods. Flowering continued until the plants were harvested. No correlation could be found between different concentrations of NaCl used and the time of flowering, flowers being produced irregularly throughout the series from the beginning of the flowering period to harvesting time. The effect of NaCl on the roots of the pea plants in the jars containing the higher concentrations of NaCl was obvious from the beginning of the experiment. Five days after setting out the plants it was noticed that in the solution containing 11,000 p.p.m. NaCl, the plants had produced very few lateral roots and in the solution containing 13,000 p.p.m., practically no lateral roots were produced. The plants in the solution containing 15,000 p.p.m. NaCl had produced no new roots, although the tops were growing. Later it was noticed that in

solutions containing 6000 p.p.m. NaCl and above, the roots were swollen in a peculiar manner. The condition of the roots in solutions of 11,000, 13,000, and 15,000 p.p.m. NaCl remained thus until the death of the plants. The swollen condition of the roots goes so far as to manifest itself in an apparent bursting of the tissue so as to show longitudinal cracks in the roots. Other investigators have referred to this peculiar condition of plant roots grown in solutions of high osmotic pressure. The seedlings in solutions of 20,000 p.p.m. NaCl and above died within a few days. Two weeks after planting, the seedlings in solutions containing 18,000 p.p.m. NaCl and above were dead, not having made any growth, whereas with few exceptions, seedlings in concentrations of 15,000 p.p.m. NaCl and below were green, and had made visi-

TABLE 9  
*Tolerance of pea plants for NaCl*

The number of plants in the various concentrations dying before completion of experiment

TIME FROM PLANTING	CONCENTRATION IN P.P.M. NaCl						
	5000	6000*	7000*	9000	11000	13000	15000
<i>days</i>							
10—							
15—							
20—							
25—						8	6
30—							
35—				2	7	6	10
40—	1	1	2	3			
45—					All plants dead		
50—		5	1	6			
55—			2				
60—							

Eighteen seedlings originally in each solution.

\* In these two concentrations 3 of the plants recorded in each were in one jar. Their death was evidently due to a factor common to the jar.

ble growth. The difference in behavior between the seedlings in concentrations of 15,000 and 18,000 p.p.m. NaCl was distinct throughout the two sets and showed a critical point above which plants could not survive under these experimental conditions, but below which growth was possible. Plants placed in some concentrations below this critical point died after making a certain amount of growth. Table 9 shows the number of plants that did not survive the various concentrations up to the time of harvesting. Those data indicate that the plants in concentrations of 11,000 p.p.m. NaCl and above died within forty days after planting. The stems of these plants increased in length and the number of leaves increased, but the change in weight was insignificant. Unlike those of the barley, the leaves of these plants withered from the edges inward just before the plants were harvested. All

plants in concentrations of 9000 p.p.m. NaCl died at approximately the same time throughout the jars of that concentration. This emphasizes again the point which we stressed in connection with the barley and the wheat series, namely, that no one part of a plant's growth period constitutes a reliable criterion for any environmental effect. Before the plants in 9000 p.p.m. NaCl died, their tops had grown 5 inches and the roots 7 inches. The plant had flowered and small pods about one inch in length had been produced.

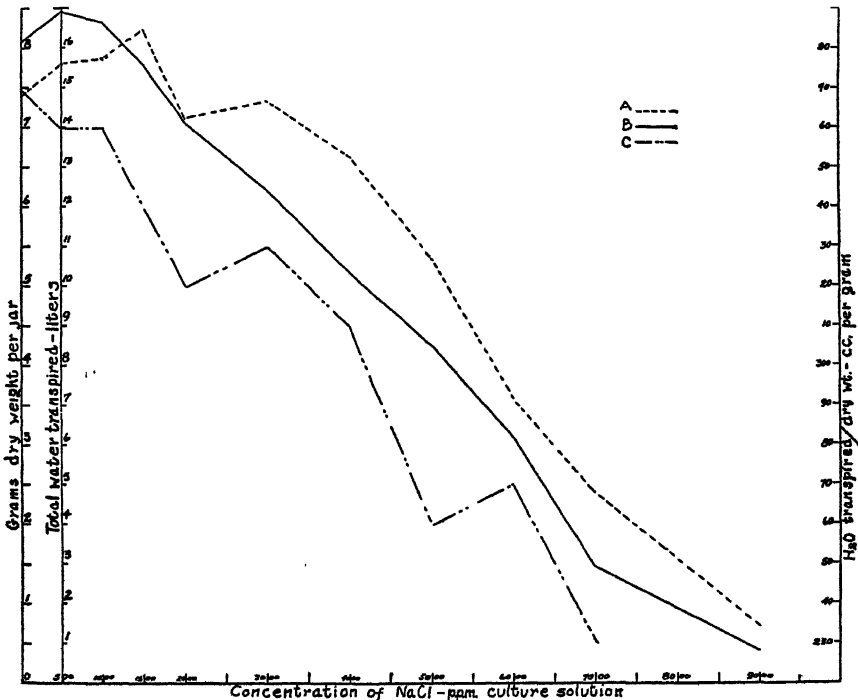


FIG. 2. THE EFFECT OF VARIOUS CONCENTRATIONS OF NaCl ON THE GROWTH OF GARDEN PEAS

A = total dry weight; B = total water transpired; C = ratio of water transpired to dry weight.

The deaths of a number of plants at lower concentrations may safely be ascribed to individual variations.

Table 8 also sets forth the results obtained in the pea series on dry weights of tops and roots, and indicates the water transpired by the plants. The relationships between the water transpired and the dry weights obtained are plotted in figure 2. Because of the great individual variability of pea plants, which is characteristic as far as is known, the probable errors of the results are high. It will be seen, nevertheless, that up to concentrations of 4000 p.p.m. NaCl, there are no significant differences in the dry weights among the



several cultures. Obviously, the differences might have been shown to be greater if the variability had not been so great. No definite relation seems to have subsisted between the tops and the roots produced in the concentrations of NaCl employed in the cultures. The curve for the water transpired follows in general the curve for total weight, but the correlation holds only within certain limits, since the former curve falls off more rapidly than the latter. In figure 2 (curve C) the transpiration coefficient is plotted against the concentrations of NaCl employed. It is seen from this that within the large area involved, the coördinates lie near a straight line.

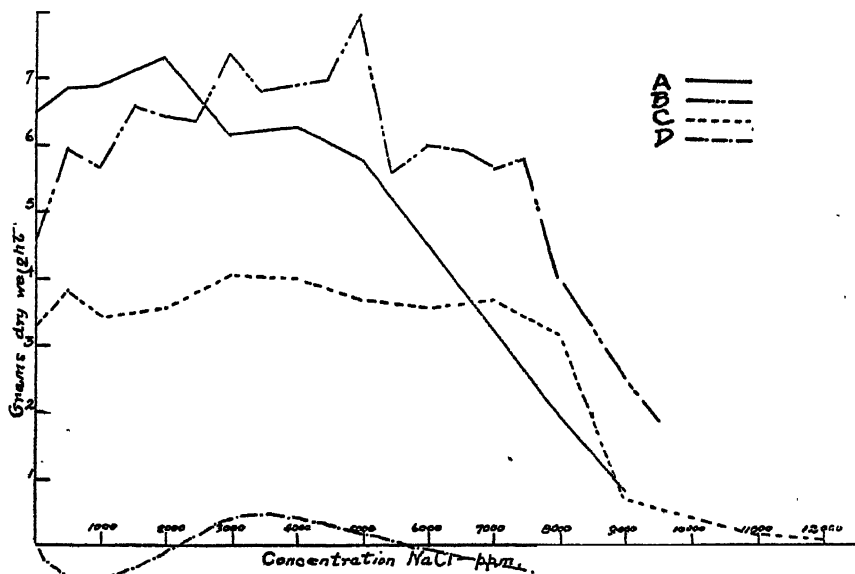


FIG. 3. A COMPARISON OF THE EFFECT OF VARIOUS CONCENTRATIONS OF NaCl ON THE GROWTH OF WHEAT, BARLEY AND PEAS

A = peas; B = wheat; C = barley, D = relative length of wheat roots after 3 weeks (diagrammatic).

As has already been indicated, the variability which characterizes pea plants does not permit us to say definitely whether concentrations of NaCl up to and including 3000 p.p.m. are stimulating to pea plants. There would seem to be some slight evidence that up to and including concentrations of 1500 p.p.m. NaCl, a slight stimulation does, nevertheless, exist. It is perfectly clear, on the other hand, that irrespective of the variability, the point of incipient toxicity of NaCl is marked with a fair degree of clearness. It appears to be at a concentration of 4000 p.p.m. NaCl. Increases of salt above that figure rapidly decreased to a marked degree the growth of the pea plants until at a concentration of 9000 p.p.m. NaCl, very little dry weight

of pea plants was produced. This also indicates that the pea plant is much more susceptible to the toxic effects of NaCl than barley or wheat plants, inasmuch as the toxicity sets in at lower concentrations of the salt. In fact, it would seem from results obtained from these series conducted under approximately the same environmental condition, that the point of definite toxicity of NaCl for pea plants is at a concentration of only slightly more than half that for barley or wheat. A graphic comparison of curves obtained for wheat, barley, and peas, is shown in figure 3. The relative length of roots in the wheat series is given by a diagrammatic curve based on observations taken at the end of the first 6 weeks of growth. Other features of the data in tables 8 and 9 require no comment.

#### GENERAL DISCUSSION

In all the experiments which have been described in this paper, including the wheat, the barley, and the pea, the toxic effects of NaCl, when they set in, manifested themselves with a fair degree of sharpness. For example, none of the plants grew to any appreciable extent above a concentration of 15,000 p.p.m. NaCl. On the other hand, with very few exceptions, growth did take place to some slight extent in a concentration of 15,000 p.p.m. NaCl. Our results, moreover, render it very clear that under controlled experimental conditions in which soil factors, including antagonism between ions, are eliminated from consideration, grasses may be stimulated by large concentrations of NaCl and may be unaffected by even larger concentrations. Although the peas do not manifest so high resistance and probably little or no stimulation from the effects of NaCl, they do nevertheless, exhibit a resistance to fairly large quantities (about 3000 p.p.m.). As has been noted, with one and the same plant in an experiment carried on at different parts of the year, results might show variations. This striking situation was brought home to the authors in subsequent experiments, which are not described in this paper, in which barley and wheat were grown outdoors during the summer season and in which none of the stimulating effects which are described above were noted, even though the resistance to the effects of high concentrations of NaCl was similar to that noted in the experiments described in this paper. The nature of the climatic complex in the environment would, therefore, seem to play a rôle of the greatest importance in determining the effectiveness of another environmental factor, as in the case of NaCl in the root medium. It is not difficult to guess at a possible explanation for this difference in the effectiveness of an environmental factor on a given plant under different climatic complexes. For example, any temperature-humidity-light complex which is favorable to a high transpiration would obviously improve the chances for a large absorption of NaCl. This would result in a marked change in the qualitative nature and concentration of the cell sap, and one would, therefore, expect that both photosynthesis and the normal metabolism of the cells would be markedly influenced thereby in the direction of depressing the

total weight. On the other hand, with a temperature-humidity-light complex in which transpiration was relatively low, the absorption of NaCl by the cells would be relatively low, and hence very different conditions would regulate photosynthesis and metabolism in the cells. Whether this one of the several possible explanations for the facts which are noted is correct, it is emphatically true that the climatic complex is an important determinant of the results that can be obtained with the same plant in experiments in which other factors are perfectly controlled. To show how striking the discrepancy between different sets of results on the same plant may be, one of the authors will publish later the results obtained by him with wheat and barley plants grown at a different season of the year but otherwise under similar conditions to those used above. However that may be, some of the higher green plants seem to possess a great resistance to NaCl and the effects which are so frequently found in alkali soils with relatively small concentrations of NaCl must evidently be ascribed not to the direct effects of NaCl but to some indirect effects exerted by it on the physico-chemical condition of the soil. The hydrogen-ion concentration of the culture solution was apparently a factor of no significance in connection with the results obtained, inasmuch as it varied fairly uniformly throughout the series, and, although started at a point approximately pH 4.9, always reached a pH of 6.7 in a relatively short time and maintained that concentration throughout the growth period of the plants.

#### SUMMARY

1. A study was made in solution cultures on the effects of a wide range of concentrations of NaCl on the growth of wheat, barley, and peas. Cultures were sufficiently replicated to control errors due to variability.
2. All plants tested show a very high resistance to NaCl.
3. Under some environmental conditions, NaCl is highly stimulating to wheat, even at concentrations of 4000 p.p.m. or more.
4. Small concentrations of about 500 to 1000 p.p.m. NaCl may depress growth particularly in the early stages, but higher concentrations may stimulate it.
5. All plants tested may make growth, though it be arrested with very high concentrations of NaCl, even up to 10,000 p.p.m. or more.
6. The concentration of NaCl at which most marked depression occurs is about 8000 p.p.m.
7. Environmental conditions are a very important determinant of the kind of results which can be obtained.
8. Some of the most striking features of these investigations can be noted only by a careful study of the whole paper.

#### REFERENCE

- (1) MUENSCHER, W. C. 1922. Effect of transpiration on absorption of salt by plants. *Amer. Jour. Bot.* 9: 311-329.

# THE ORIGIN AND NATURE OF THE SOIL ORGANIC MATTER OR SOIL "HUMUS": III. THE NATURE OF THE SUBSTANCES CONTRIBUTING TO THE FORMATION OF HUMUS<sup>1</sup>

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The great majority of investigations dealing with the subject of transformation of organic matter in the soil have been concerned either (a) with the decomposition of a single purified material, whereby one or more of the intermediary or final products of the reaction were measured, or (b) with the change into "humus" of some complex natural organic substance such as cereal straw, corn stover, legume hay, or wood products.

The origin of this so-called "humus," or alkali-soluble organic matter, in the soil is still the subject of various hypotheses, most of which are based upon few experimental facts. On the one hand "humus" was believed to be formed in the soil as a result of "humification" processes carried on by various groups of microorganisms; the nature of the organisms and the chemistry of the processes being very vague. On the other hand, some investigators (6, 7) found that the organic matter added to the soil already contains alkali-soluble material, which actually decreases quantitatively in the soil as a result of decomposition. These investigators claim that there is no specific "humification" in the soil, that the "humus" is actually added to the soil, that it is not formed there, and that it is even gradually decomposed in the soil.

With the exception of a few final products, such as ammonia accumulated and carbon dioxide evolved, very little is known concerning the numerous changes whereby rapidly decomposing natural organic substances are transformed, finally yielding the more or less resistant "humus" materials. This is due largely to the fact that, in the great majority of investigations, a single process has been studied, such as ammonia formation or carbon dioxide evolution, sometimes carried on by a single organism, when the latter was considered at all. Often the interest centered entirely upon the fate of a single elemental constituent of the organic matter, usually nitrogen. The various terms of "decay," "humification," "putrefaction" do not stand for any definite chemical or biological processes but merely for certain sets of condi-

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tions leading to the formation of certain complex preparations. They are, therefore, valueless in attempting to present a scientific interpretation of the chemical or biological transformation of organic matter in the soil, which results in the formation of dark colored substances referred to collectively as "humus."

It is essential to know the fate of every ingredient of the organic matter added to the soil, the various chemical processes through which it changes, and to what extent it contributes to the soil organic matter. After the information concerning the transformation in the soil of the various carbohydrates, proteins, fats, and other constituents of the natural organic matter is available, an attempt should be made to learn how the presence of one substance influences the decomposition of another. A study of the decomposition of the natural organic matter itself could then be undertaken. Only when the processes thus involved are understood, when the various intermediary, final, and transformation products are known, can one expect to learn the nature of the soil organic matter. It has been shown, for example (8), that in the decomposition of celluloses by filamentous fungi, no residual substances are left which could be classified under "humus." About 60 to 65 per cent of the carbon of the celluloses decomposed was liberated as  $\text{CO}_2$  and about 30 per cent of the carbon was reassimilated by the organisms active in the decomposition of the celluloses, in the synthesis of their protoplasm. It was suggested that this synthesized mass of organic matter contributes to the soil "humus." Celluloses are thus found not to play any direct part in the formation of soil "humus." Indirectly, however, they, as well as other carbon compounds offering available sources of energy, contribute decidedly to the soil "humus." The decomposition of celluloses under anaerobic conditions is carried out entirely by bacteria, with the formation of various organic acids and the synthesis of a much smaller amount of protoplasm (12). Under aerobic conditions, celluloses may be decomposed by bacteria with the formation of hemicelluloses, pigments (of the carotin group), and bacterial cells (9). The rôle of celluloses in the formation of "humus" thus depends also upon the environmental conditions under which the decomposition takes place. A knowledge of the chemical composition of the organisms concerned in the decomposition of the organic substances, of the mechanism whereby they obtain their energy, of the efficiency of utilization of the available energy, of the nature of assimilation of the nitrogen and minerals necessary for the synthesis of their cells, and of the relation between the available energy and nitrogen transformation, is, therefore, essential. It has been shown, for example (5, 11), that the formation of ammonia from pure nitrogenous substances by pure or mixed cultures of microorganisms depends not only upon the nitrogen content of the substance in question but also upon the ratio between the available energy (carbon) and the nitrogen content. Substances with a narrow C:N ratio gave much more ammonia than substances with a wide C:N ratio.

When fresh organic matter, in the form of straw, green manure, various plant and animal products, or plant stubble is added to the soil, decomposition sets in rapidly. The total rate of the processes of decomposition can be most conveniently measured by the rate of evolution of  $\text{CO}_2$ . If the organic material is rich in proteins, the rate of formation of ammonia or the accumulation of ammonia and nitrate nitrogen may also be used in following the rate of decomposition. Not all the organic matter is decomposed in the soil, since both the  $\text{CO}_2$  and the final nitrogenous substances (ammonia and nitrate) account for only a part of the original material added. A definite part of the organic matter is reassimilated by the microorganisms active in the processes of decomposition. A part of the original organic matter may be left in the form of various intermediary products such as organic acids and higher alcohols, especially under anaerobic conditions, but this part will tend not to persist in the soil and will be further decomposed in the presence of sufficient base.

TABLE 1

*Composition of some natural organic materials on the basis of water-free matter (after Pringsheim)*

SUBSTANCE	ASH	FATS AND WAXES	CRUDE PROTEIN	PENTO-SANS	CELLULOSE PURE	LIGNIN (WILL-STÄTTER METHOD)
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Winter straw.....	4.33	0.67	3.00	21.67	34.27	21.21
Summer straw (oats).....	4.81	2.02	4.70	21.33	35.43	20.40
Corn stover.....	6.15	0.77	3.50	23.54	30.56	15.13
Corn cobs.....	1.80	1.37	2.11	31.50	37.66	14.70
Hay.....	6.05	2.00	9.31	13.52	28.50	28.25
Reeds.....	5.79	0.91	3.76	20.94	33.35	20.33
Pine wood.....	0.53	3.17	1.27	10.80	41.93	29.52

There is another part of the natural organic matter left in the soil, namely, a part of the original material which resists decomposition. It is this undecomposed organic matter, the various intermediary products, and the newly synthesized materials which go to make up the soil organic matter or "humus."

To understand the nature of this "humus," it is essential to know the chemical nature of the various substances which go to make up the original organic matter added to the soil, the processes which each of the chemical constituents undergoes in the soil, the environmental conditions, and the nature of the microorganisms bringing these processes about. Since the organic materials added to the soil are very complex and consist of as many chemical substances as only the complex protoplasm of the different cells of plants, animals, and microorganisms can be expected to contain, and in view of the fact that the soil harbors a great many types of microorganisms varying in their metabolisms, many of which contribute to the transformation of the soil organic matter, one can readily understand that the problem under consideration is not very simple.

Of the different ingredients of the natural organic matter of plant origin commonly added to the soil, it is sufficient to consider, for the present, the transformation of the celluloses, starches, pentosans, and other polysaccharides and monosaccharides, which make up about 40 to 70 per cent of the dry matter; proteins and protein derivatives (1 to 20 per cent); the lignins (10 to 30 per cent); and the ether- and alcohol-soluble substances or fats and waxes (0.5 to 4 per cent), as shown in table 1.

Various natural organic materials also contain soluble sugars, Collison (3) having reported 1.56 per cent reducing sugar in wheat straw, 1.38 per cent in corn stover, 5.97 per cent in timothy hay, and 4.35 per cent in alfalfa. The amount of sugars as well as of starches and pectins depends not only upon the nature of the plant but also upon the degree of its maturity.

The problem of the origin of "humus" in the soil thus reduces itself to the following questions:

1. To what extent do the natural organic substances contribute directly, in the form of various ingredients, to the soil "humus"?
2. What are the chemical and biological processes whereby the natural organic substances are decomposed, and how do these contribute to the soil "humus"?
3. What rôle do microorganisms play in the transformation of energy in the soil and how much of this energy is stored away in newly synthesized protoplasm? What relation does this synthesized protoplasm have to the soil "humus"?
4. How do the activities of the microorganisms influence the composition of the "humus," especially its carbon and nitrogen relationship?

#### EXPERIMENTAL

To determine what constituents of natural organic matter are first decomposed by microorganisms and what constituents resist decomposition, a series of experiments was undertaken on natural organic matter, from which one or more fractions had been removed. The methods outlined by Dore (4), Johnsen and Hovey (10) and others, on wood analysis, were utilized both for removing various ingredients of certain natural products, such as straw, and for measuring the amount of decomposition of each of these ingredients. Cellulose in pure form was determined by the method of Charpentier (2), and cellulose in the form of natural materials was determined by the method of Bengtsson (1). Lignin was determined by the method of Willstätter (14). The alkali-soluble portion of natural organic materials precipitated with hydrochloric acid is often considered as lignin. This preparation, however, contains a considerable amount of pentosans (xylans), which may or may not be utilized as sources of energy by different organisms. These pentosans can be removed, if the precipitate is boiled in the presence of an excess of 2 to 3 per cent acid. Ammonia was determined by leaching the soil with normal KCl solution, then washing with water and distilling the extract with some heavy MgO into standard acid solution.

A series of 10-gm. portions of a uniform quantity of finely ground rye straw was subjected to a series of treatments, at every step two portions of the

treated straw being left for the study of its decomposition by microorganisms. The results of the analyses of this straw by the methods adapted are given in table 2.

An attempt was first made to study the decomposition of various straw preparations by pure cultures of soil organisms. To select a proper organism which would be capable of attacking vigorously the various constituents of the straw, the following preliminary experiments were carried out:

In 300-cc. round bottom flasks, 100-gm. portions of washed white sand were placed; 3-gm. portions of the straw preparations and 25 cc. of a nutrient solution, containing 10 gm.  $(\text{NH}_4)_2\text{HPO}_4$ , 2 gm. KCl, 2 gm.  $\text{MgSO}_4$ , and 0.02 gm.  $\text{FeSO}_4$  in 1000 cc. of distilled water were added to each flask, which was then plugged with cotton and sterilized, at 15 pounds pressure for 1 hour. The flasks were inoculated in duplicate and connected with a respiration apparatus (13), which was kept in the thermostat at  $28^\circ\text{C}$ . The following organisms were tested: *Bac. cereus*, a cellulose-decomposing bacterium No. 7, *Zygorhynchus mölleri* and a green *Trichoderma*. The carbon dioxide produced in the decomposition of the

TABLE 2  
*Composition of rye straw*

	per cent
Moisture.....	6.37
Loss on extraction with benzene and alcohol.....	3.23
Loss on extraction with cold water for 24 hours.....	7.01
Loss on extraction with 5 per cent NaOH for 24 hours in cold.....	29.44
(NaOH extract precipitated with HCl, but not boiled in excess of acid.....	22.33)
(Lignin obtained directly by Willstätter method.....	18.79)
Loss on extraction with 2 per cent sulfuric acid by boiling 2 hours.....	21.15
Residual material (largely cellulose).....	32.36
Total.....	99.56

straw was absorbed in standard barium hydroxide solution, which was titrated back, as often as necessary, with standard oxalic acid solution.

At the end of 21 days incubation, the flasks were disconnected and the residual ammonia, residual organic matter, and cellulose were determined in aliquot portions. The residual organic matter was obtained by washing off all the organic matter from the sand upon a weighed filter paper, then drying the paper and contents to constant weight, igniting, and weighing again. The loss in weight indicates the total amount of organic matter left, free from moisture, of the water-soluble fraction and of ash. (Table 3.)

The results indicate quite definitely that *Trichoderma* and the Bacterium No. 7 are organisms very active in the decomposition of organic matter. The fungus decomposed more of the cellulose as well as more of the total organic matter than the bacterium. It is interesting to note, however, that the latter produced more carbon dioxide than the fungus. This is no doubt due to the fact that the *Trichoderma* assimilated more of the carbon for the synthesis of its more extensive protoplasm than the bacterium. This can be seen readily from the amount of residual ammonia: although the bacterium liberated 211.95



mgm. of carbon as carbon dioxide and decomposed at least 708 mgm. of water-insoluble organic matter, it assimilated only 17.0 mgm. of ammonia-nitrogen, showing a ratio of 12.5 between the carbon given off as carbon dioxide and the nitrogen assimilated. The fungus liberated only 188.71 mgm. of carbon as carbon dioxide but it decomposed 1228 mgm. of water-insoluble organic matter and assimilated 25.5 mgm. of ammonia-nitrogen, with a ratio of 7.5 between the carbon of carbon dioxide liberated and the nitrogen assimilated. The ratio between the water-insoluble organic matter decomposed and the ammonia-nitrogen assimilated is about the same in both cases, the difference being largely in the amount of carbon liberated as a waste product. This indicates that the bacterium consumed a considerably larger amount of carbon in proportion to the amount of nitrogen assimilated than did the fungus. The fact that bacterial cells are usually richer in nitrogen than the fungus mycelium tends further to emphasize the greater consumption of energy per unit of quantity of cell material synthesized in the case of bacteria than in the

TABLE 3  
*Decomposition of straw by different microorganisms in sand media*

NAME OF ORGANISM	CO <sub>2</sub> PRODUCED, AS CARBON									RESIDUAL AMMONIA (N)	RESIDUAL ORGANIC MATTER*	RESIDUAL CELLULOSE
	2 days	4 days	5 days	8 days	10 days	12 days	14 days	21 days	Total			
	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Control.....	1.97			2.11				5.76	9.84	45.8	2,336	1,020
<i>Bac. cereus</i> .....	2.59	19.15		9.50				26.21	57.45	44.9	2,308	1,016
<i>Bacterium</i> No. 7	2.30		12.96	43.20	27.79	27.36	26.92	71.42	211.95	28.8	1,628	778
<i>Zygorhynchus</i> ...	20.45	8.92	10.66	8.35				30.10	78.48	45.8	2,108	1,024
<i>Trichoderma</i> ....	21.17	51.64	25.34	39.31	13.10	8.78	12.81	16.56	188.71	20.3	1,380	622

\* Free from moisture, water-soluble substances, and ash.

case of fungi. There is ample evidence in the literature to indicate that fungi are much more efficient than bacteria in the utilization of energy. The assimilation of 25.5 mgm. nitrogen by the fungus indicates that at least about 500 mgm. of mycelium has been synthesized, assuming that the nitrogen content of the mycelium is about 5 per cent. This considerable quantity of synthesized mycelium (except the water-soluble portion) is included in the residual organic matter. The fact that fungi take an active part in the decomposition of celluloses in the soil (12), and the fact that they may re-assimilate 30 parts of the carbon of the celluloses and other energy sources decomposed, point to the development of these organisms as an important source of "humus" in the soil.

The *Zygorhynchus* did not attack the celluloses; its activities seemed to have been limited to the soluble carbohydrates and proteins of the straw, as indicated by the fact that it did not assimilate any of the ammoniacal nitrogen. The same is true of the *Bac. cereus*.

To obtain further information concerning the ability of different organisms to attack straw and certain of its constituents, the above experiment was repeated, only 5 gm. of  $(\text{NH}_4)_2\text{HPO}_4$  being used per liter of medium and 25 cc. of the nutrient solution being added to 100-gm. portions of washed sand containing 3 gm. of rye straw. The cultures were sterilized, inoculated with pure cultures of the different organisms, and incubated for 30 days. The determinations were then made as in the previous experiment, and the results are recorded in table 4.

The results show definitely that *Trichoderma* is most active in decomposing the straw as a whole, nearly 800 mgm. of the water-insoluble, ash-free organic matter being decomposed in 30 days. This is accompanied by a considerable absorption of ammonia nitrogen, which has become transformed into microbial protoplasm. The *Zygorhynchus* consumed a much smaller amount of the organic matter, accompanied also by a very low ammonia assimilation. The

TABLE 4  
*Decomposition of straw by pure cultures of organisms.\**

ORGANISM	AMMONIA (N)		RESIDUAL ORGANIC MATTER LEFT	
	Left in 100 gm. of sand medium	Used up by organism	Total organic matter, free from ash and water-soluble constituents	Precipitate formed by treating NaOH solution with excess of acid
	mgm.	mgm.	mgm.	mgm.
<i>Trichoderma</i> .....	8.4	16.0	1,932	324
<i>Zygorhynchus</i> .....	22.1	2.3	2,388	490
<i>Actinomyces viridochromogenus</i> .....	21.4	3.0	2,193	286
<i>Bacillus cereus</i> .....	19.4	5.0	2,657	496
Control.....	24.4	0	2,729	504

\* Three grams of straw, containing about 2700 mgm. of dry matter free from ash and water-soluble constituents, added to 100 gm. of white, washed sand.

*Bac. cereus* decomposed only a small amount of the straw, probably only the reducing sugars and certain readily soluble substances. The actinomyces decomposed a considerable amount of the straw (536 mgm.), but it assimilated only a small amount of nitrogen. The decomposition of the fraction of the straw soluble in alkalis and precipitated by hydrochloric acid is of especial interest. This fraction comprises lignins and certain pentosans (the preparation not being boiled in acid, for these experiments). The *Zygorhynchus* and the bacterium practically did not attack this fraction at all. The *Trichoderma* decomposed about one-third of it; this action was due to the presence of pentosans which were brought down with the lignins in the hydrochloric acid precipitate of the alkaline extract of the organic matter. The actinomyces decomposed considerably less total organic matter than the *Trichoderma* but a larger part of the fraction soluble in alkalis and precipitated by

acids. This is a result of the fact that this organism is actually capable of decomposing lignins in nature, as will be shown in a later contribution.

As a result of these and other experiments, the *Trichoderma* was selected for the following investigation:

A series of 10-gm. portions of rye straw were treated in a manner outlined in table 2. All were extracted first with benzene and then, after the removal of the benzene, with 95 per cent alcohol. Two portions of the straw thus treated were left as preparation 2. The other portions were treated, after the removal of the alcohol, with cold water for 24 hours, then filtered and dried. Two portions were again set aside, as preparation 3. Several of the remaining portions were treated according to the method of Willstätter (14) so as to obtain lignin directly. The other portions were extracted, at 15 pounds pressure for 30 minutes, with 50-cc. portions of 5 per cent sodium hydroxide solution, filtered, washed with water, then with dilute acetic acid, again with water, and then dried. Two of the portions thus treated were set aside as preparation 4. The remaining portions were boiled with 200 cc. of 2 per cent sulfuric acid solution for 2 hours, then filtered and washed, to give preparation 5. The lignin prepared according to the method of Willstätter formed preparation 6. The alkaline extract of the straw was neutralized with hydrochloric extract; the precipitate was filtered, washed with water and dried, to give preparation 7; it consisted partly of lignin and partly of pentosans. The untreated straw formed preparation 1. Sixteen 100-gm. portions of a Sassafras sandy soil and 16 portions of washed white sand were placed in 300-cc. long-necked flasks. Two-gram portions of preparations 1 to 5 and 1.5-gm. portions of 6 and 7 were added to the soil and sand flasks, leaving two flasks in each series as controls. Twenty-five-cubic centimeter portions of the first nutrient medium were added to all the flasks, which were then plugged and sterilized. The soil flasks were inoculated with a crude culture of cellulose-decomposing bacteria, containing also *Bacterium* No. 7 and protozoa, but free from fungi and actinomycetes. The sand flasks were inoculated with a pure culture of the *Trichoderma*. The flasks were then connected with the respirator and incubated at 25 to 30°C. for 31 days. At the end of the period of incubation the residual ammonia and "humus" were determined; the latter was measured by extracting the soil or sand culture twice with sodium hydroxide, then precipitating the extract with hydrochloric acid. The "humus" given in table 5 represents the sum of the  $\alpha$ -fraction and the organic matter of the  $\beta$ -fraction.

The results obtained are instructive. They show first of all that pure lignin is not utilized either by strong cellulose-decomposing bacteria or by fungi. When the lignin prepared by the Willstätter method was used, there was actually no trace of decomposition; when that part of the alkali extract which is precipitated with hot hydrochloric acid was used, some decomposition took place because of the presence of pentosans in the preparation.

The results further show that the lignin is found in the same preparation, obtained by the same methods commonly used for determining "humus" and "humic acids;" the lignin which accumulates in the soil forms at least a part of this "humus" or "humic acids." Practically all the lignin introduced with the straw or with the variously treated straw preparations is obtained again almost quantitatively in the final "humus." Preparations 4 and 5 from which most of the lignin has been removed by treatment with sodium hydroxide gave in the soil and sand media a much smaller amount of additional "humus" than preparations 1 to 3 from which the lignin has not been removed. The fact that there is an actual increase in the "humus" above that part which can be accounted for by the lignin shows another phenomenon, which will be brought

TABLE 5  
 Decomposition of straw and its constituents by the soil population and by *Trichoderma*

PREPARA- TION NUMBER*	AMOUNT OF MATERIAL ADDED TO 100 GM. OF SOIL OR SAND	MEDIUM†	CO <sub>2</sub> LIBERATED AS C								NH <sub>4</sub> -N LEFT‡	NH <sub>4</sub> -N USED UP	"HUMUS" CONTENT OF SOIL	EXCESS "HUMUS" OVER CONTROL		
			2 days	4 days	7 days	13 days	21 days	31 days	Total	Total— control						
Control																
1	2	Soil	8.43	7.45	5.49	18.71	13.72	16.36	70.16		49.8	20.7	2,488	479		
2	2	Soil	13.82	16.37	23.42	61.74	46.45	37.30	199.10	128.94	29.1	21.0	2,967	499		
3	2	Soil	20.68	18.33	28.02	37.35	26.45	40.28	171.12	100.96	28.8	20.7	2,989	368		
4	2	Soil	14.11	14.61	17.54	54.70	29.20	37.30	167.46	97.30	30.9	18.9	2,756	224		
5	2	Soil	12.05	12.05	11.00	31.28	58.90	42.06	167.34	97.18	35.4	14.4	2,712	231		
6	1.5	Soil	15.97	8.82	12.54	33.12	32.06	47.04	149.55	79.39	34.2	15.6	2,719	1,130		
7	1.5	Soil	6.86	5.19	13.13	19.40	22.94	17.16	72.05	1.89	46.9	3.0	3,618	896		
Control			7.55	16.37		19.80	22.14	19.20	98.19	28.03	44.4	5.4	3,084	0		
1	2	Sand	1.28				4.70	12.84	18.82		45.2			346		
2	2	Sand	29.01	41.06	18.92	20.28	14.12	13.52	136.91	118.09	29.4	15.8		364		
3	2	Sand	21.27	33.61	22.84	22.05	12.84	12.25	124.86	106.04	28.5	16.7		392		
4	2	Sand	21.96	50.18	29.01	10.88	33.32	17.45	162.80	141.98	25.8	19.4		135		
5	2	Sand	17.06	28.71	42.92	24.80	41.75	18.23	173.47	154.65	19.5	25.7		150		
6	2	Sand	9.70	12.35	32.15	63.42	29.40	20.78	167.80	148.98	23.4	21.8		1,287		
7	1.5	Sand	3.04				5.00	5.20	13.24	-5.58	45.0	0.2		816		
	1.5	Sand	2.06	14.70	5.59		7.45	4.12	33.92	15.10	43.5	1.7				

\* The preparation of the straw constituents is given in table 2; no. 1 is straw itself; no. 2 is straw from which the benzene- and alcohol-soluble fractions have been removed; no. 3 is straw from which the benzene- and alcohol- and cold water-soluble fractions have been removed; no. 4 has also the NaOH-soluble portion (5 per cent NaOH solution used under pressure) removed; no. 5 has also the rest of the pentosans removed, by boiling for 2 hours with 2 per cent H<sub>2</sub>SO<sub>4</sub>; no. 6 is lignin prepared by Willstätter method; no. 7 is the precipitate obtained by neutralizing the NaOH extract with HCl, washing and drying.

† Soil medium: 100 gm. of dry soil with 25 cc. nutrient solution sterilized and inoculated with crude suspension of cellulose-decomposing and other bacteria, and protozoa (no fungi or actinomycetes). Sand medium: 100 gm. of washed sand with 25 cc. of nutrient solution sterilized and inoculated with *Trichoderma*.

‡ Control soil contains 4.6 mgm. NH<sub>4</sub>-N.

out in detail later, namely, that a part of the 'humus' is actually synthesized in the soil by the action of microorganisms. In other words, the lignin contributes a part of the "humus" (not only quantitatively but also qualitatively) and the synthesized cells of the microorganisms contribute another part. The synthesizing action of the microorganisms is clearly brought out by the quantities of ammonia used up and converted into microbial protoplasm.

It is interesting to compare the action of the bacteria in the soil and of the fungus in the sand upon the different straw preparations. The strong cellulose-decomposing fungus makes a better growth, as indicated by the increase in  $\text{CO}_2$  evolution and the ammonia consumption, with an increase in cellulose and pentosan content of the preparation (4 and 5). The bacteria, however, thrive better on the alcohol-, water-, and alkali-soluble extractives, although some of the celluloses and pentosans are also decomposed. This is in conformity with studies reported elsewhere (12) that the celluloses in normal humid soils are largely decomposed by the filamentous fungi of the soil.

Without going into a detailed discussion of the rate of decomposition of the various constituents of the straw, which is left for the following contribution where more extensive data are presented dealing with this subject, it is sufficient to point out the fact that both the evolution of  $\text{CO}_2$  and the transformation of nitrogen from an inorganic into an organic form point to a much greater decomposition of the celluloses by the fungus when the lignins have been removed. In other words, the lignin in the straw not only does not decompose but it even prevents to a certain extent the decomposition of the celluloses and hemicelluloses. If  $\text{CO}_2$  can be looked upon as a true index of the respiration of an organism and if the same organism produces the same amount of  $\text{CO}_2$  when different carbohydrates are used as sources of energy, untreated straw is decomposed considerably slower than the straw from which the fats and waxes, water-soluble and alkali-soluble portions have been removed. The ratio between the carbon liberated as  $\text{CO}_2$  and the nitrogen assimilated is very narrow in the case of the pure fungus culture, being about 7.5 for the untreated straw and about 7.0 for the cellulose obtained from this straw. This fact serves to illustrate what extensive quantities of nitrogen are required by the organism when straw is plowed under. The undecomposed straw will serve to keep the soil at a point of nitrogen starvation as far as higher plants are concerned as long as there is an excess of available energy.

#### SUMMARY

Among the various ingredients of natural organic material, such as straw, the lignins are most resistant to the action of fungi and bacteria; their accumulation in the soil accounts for a large part of the soil "humus," which is formed as a result of the decomposition of the straw. Since "humus" is usually considered as that part of the soil organic matter which is extracted by alkalis and precipitated by acids, the lignins added to the soil form one of the constituents of this "humus." This is due to the fact that most of the fungi and

bacteria which attack the natural organic substances added to the soil are unable to decompose lignins to any considerable extent; the lignins are thus allowed to accumulate in the soil, in the absence of organisms which are capable of decomposing them. The nature of these organisms will be discussed in detail later.

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# INFLUENCE OF SULFUR AND GYPSUM ON THE SOLUBILITY OF POTASSIUM IN SOILS AND ON THE QUANTITY OF THIS ELEMENT REMOVED BY CERTAIN PLANTS<sup>1</sup>

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## HISTORICAL

Ames and Boltz (1) have shown that sulfur, alone or in connection with other treatments, increased the water-soluble potassium in some Ohio soils, but they attribute the benefit to salt action rather than to acidity generated by the oxidation of the sulfur. Later, Ames and Simon (3) found that gypsum also appreciably increased the water-solubility of potassium in some soils.

André (4) reports increased solubility of potassium in microcline in contact with gypsum or calcium carbonate.

Bradley (6) carried on experiments on some Oregon soils by permitting the moist samples to remain in contact with lime or gypsum for several weeks in some instances. His conclusions were that gypsum acts as an indirect potassium fertilizer but that lime does not, in fact, lime sometimes depressed the solubility of potassium.

Briggs and Breazeale (7) conclude that availability to plants of the potassium in soils derived from orthoclase-bearing rocks is not increased by the addition of lime or gypsum; in fact, gypsum sometimes depresses potassium solubility. Their conclusions are based both on solubility studies and on the potassium content of wheat seedlings grown in the solutions.

Burgess (9) showed that gypsum increased the water-soluble potassium in a California soil which was unproductive but it had no effect on plant growth.

Dumont (11) found that gypsum increases the water-soluble potassium in some granitic soils.

Frap (12) concludes from his solubility studies on Texas soils that lime or gypsum has very little, if any, effect on rendering potassium available to plants.

Lipman and Gericke (16) conducted experiments on three California soils with lime and gypsum. They obtained increased solubilities of potassium in water with both materials in two but not in the third soil, which was of a different type. They conclude that the apparently contradictory results found by different investigators can be attributed to the use of different types of soil in the experiments.

McCall and Smith (19) studied the effect of composting samples of Maryland and New Jersey greensands with sulfur, manure, and soil. They obtained large amounts of water-soluble potassium in some mixtures when sulfur was present. Their results indicate that their procedure may prove to be a practical and efficient method of obtaining available potassium from comparatively insoluble materials.

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McMiller (20) found that when some Minnesota soils were mixed with gypsum and then kept in a moist condition for some time, marked increases in potassium soluble in water sometimes were obtained. He attributes the negative results found by some investigators to unnatural conditions of contact of soil and gypsum.

Morse and Curry (21) report that lime and gypsum in contact with feldspar give increased water-soluble potassium but their effect is negligible in mixtures of feldspar and clay. They conclude that any solvent action which these materials might have on potassium of feldspar in a soil would be counteracted by the adsorptive property of its clay for this constituent.

Rudolfs (22) in his experiments on composting New Jersey greensand and sulfur found that small amounts of water-soluble potassium were liberated.

The foregoing references are conflicting regarding the positive action of gypsum in liberating potassium from different types of soil. Apparently, gypsum may liberate potassium in some types of soil but not in others. In view of recent experiments, the assertions made by some of the earlier writers, such as Hilgard (14, p. 379) and Storer (24, p. 207), that this material liberates potassium from soil silicates and supplies this essential ingredient in a soluble form is seen to be too general. Probably it would be more accurate to state, as mentioned by Lyon and Buckman (18, p. 379), that although gypsum has generally been credited with having the property of liberating potassium in soils, the experimental evidence is conflicting.

#### OBJECT OF THIS INVESTIGATION

The writer has been studying, for some time, the effect of S in Kentucky soils and has shown that it is readily oxidized by the sulfofying organisms of the soil to  $\text{H}_2\text{SO}_4$ . This acid then reacts with the phosphate in the soil or with  $\text{Ca}_3(\text{PO}_4)_2$  which may have been added, to form soluble P compounds (23). This fact was first announced by Lipman and co-workers (17) and was later confirmed by other investigators (2, 8).

As the work regarding the effect of S on the liberation of K in soils is meager and that concerning the action of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  is conflicting, it was thought that further studies of this character on several types of Kentucky soils might contribute something of interest to this subject.

#### PLAN OF THE EXPERIMENTS

A large majority of the experiments referred to above involved additions of rather large amounts of either S or  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  to the soil. The quantities varied from 250 to over 300,000 p.p.m. of soil and, with few exceptions, they were larger than correspond to field applications. As such large amounts are abnormal, it was decided for these experiments to employ quantities that do not greatly exceed those used in practice.

The soils selected were of different types found in Kentucky and representative of large areas. They were as follows:

Graves County. Untreated plots of experiment field. Yellowish-brown silt loam, undulating phase.

Fayette County. Untreated plots of experiment field. Light brown silt loam corresponding to Hagerstown silt loam—United States Bureau of Soils.

McCracken County. Untreated plots of experiment field. Yellowish-brown silt loam.

Taylor County. Untreated plots of experiment field. Yellowish-brown silt loam.

Shelby County. Composite of two soils from Eden formation. Light yellow silt loam.  
Franklin County. Composite of three soils from Eden formation. Medium yellowish-brown silty clay loam.

Madison County. No. 1—Composite of two soils from Eden formation. (Hagerstown stony clay—United States Bureau of Soils.) Brown clay loam.

Madison County. No. 2—Untreated plots of experiment field. (DeKalb silt loam—United States Bureau of Soils) Yellowish-gray silt loam to silty clay loam.

Muhlenburg County. Untreated plots of experiment field. (Tilsit silt loam—United States Bureau of Soils) Yellowish-brown silt loam.

Logan County. Untreated plots of experiment field. (Decatur silt loam—United States Bureau of Soils) Reddish-brown silt loam.

Laurel County. Untreated plots of experiment field. Yellowish-brown silt loam to silty clay loam.

The samples were air-dried and put through a 2-mm. sieve. Nothing was added other than the chemicals mentioned in the tables. The required amount of each chemical was intimately mixed with the dry soil after which distilled  $H_2O$  was added to the extent of 20 per cent of the weight of air-dried soil. The moist samples were in good physical condition and could be stirred without packing.

The experiments were carried on in pint glass jars which had been previously weighed and were always kept covered with watch glasses. At intervals of every two or three weeks, the necessary quantity of distilled  $H_2O$  was added to replace the small amount lost by evaporation. Each time this was done the soil was thoroughly stirred.

The quantities of S or  $CaSO_4 \cdot 2H_2O$  added were 250 p.p.m. of air-dried soil unless otherwise noted in the tables. The amount of  $CaCO_3$ , where applied, was 4000 p.p.m. The chemicals were the best C.P. grade and were previously tested to insure the absence of impurities which would affect the results.

The soils were kept in the jars, at laboratory temperature, for 4 months, at the end of which time each sample was stirred, after adding the required quantity of distilled  $H_2O$  to restore its initial moisture content, and portions were weighed out for the digestions, all of which except those mentioned later were made on the moist soil.

The determinations were total K and  $SO_4$  on the original air-dried soils and soluble K, total  $SO_4$ , and hydrogen-ion concentration on the treated soils. The last two determinations were made on the air-dried samples and the soluble K was made on the moist soils except those with 0.2N  $HNO_3$  described later.

The solubility of the K was determined by digestions in distilled  $H_2O$ , in 0.2N  $HNO_3$ , and in either 0.1M or 0.2M  $NH_4NO_3$ .

The 0.2N  $HNO_3$  digestions were made on the air-dried soil remaining after extracting with distilled  $H_2O$ . The digestions in  $NH_4NO_3$  solution were for comparison with those by  $HNO_3$  so as to determine what effect the equivalent concentration of the neutral salt solution of that acid would have on the soil.

The amounts of  $H_2O$  and  $CaCO_3$  present in the soils were taken into consideration in preparing the solvents for the various digestions so as not to affect their volume and strength.

In addition to the above, analyses were made of the content of K in young wheat and buckwheat plants grown on the soils after treatment. These will be described later.

TABLE I  
*Graves County soil—Total K, 14,200 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		<i>p.p.m.</i>	<i>per cent</i> *	<i>gm.</i>	<i>per cent</i> †		
None	Distilled H <sub>2</sub> O	19	0.13	0.0091	2.59	0.35	} Wheat
	0.2 <i>N</i> HNO <sub>3</sub>	69	0.49				
	Sum	88	0.62				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	151	1.06				
S	Distilled H <sub>2</sub> O	22	0.15	0.0058	1.88	0.31	
	0.2 <i>N</i> HNO <sub>3</sub>	66	0.46				
	Sum	88	0.61				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	305	2.15				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	19	0.13	0.0062	2.08	0.30	
	0.2 <i>N</i> HNO <sub>3</sub>	65	0.46				
	Sum	84	0.59				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	250	1.76				
CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	12	0.08	0.0162	1.46	1.11	
	0.2 <i>N</i> HNO <sub>3</sub>	65	0.46				
	Sum	77	0.54				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	56	0.39				
S and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	12	0.08	0.0157	1.45	1.08	} Buck- wheat
	0.2 <i>N</i> HNO <sub>3</sub>	66	0.46				
	Sum	78	0.54				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	59	0.42				
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	11	0.08	0.0125	1.30	0.96	
	0.2 <i>N</i> HNO <sub>3</sub>	63	0.44				
	Sum	74	0.52				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	58	0.41				

\* Of the total K.

† In air-dry plants.

#### METHODS OF ANALYSIS

*Total K.* Modified J. L. Smith method (5). In washing the fusion residue, only about 300 cc. of hot distilled  $H_2O$  was used which removed the K. The  $K_2PtCl_6$  was treated with acid  $C_2H_5OH$  (10 volumes  $C_2H_5OH$ , 95 per cent: 1 conc.  $HCl$ ) and allowed to stand over night after which the  $K_2PtCl_6$  was

filtered and washed with acid  $C_2H_5OH$ , then with the usual  $NH_4Cl$  solution, and finally with  $C_2H_5OH$  (90 per cent).

*H<sub>2</sub>O-soluble K.* To 132 gm. of the moist soil (110 gm. air-dried) 1078 cc. of distilled  $H_2O$  was added and digested for 4 days at room temperature, with shaking at 3-hour intervals each day. The whole was then transferred to a folded filter and the solution refiltered through the soil until clear. A liter of the filtrate, equal to 100 gm. of air-dried soil, was evaporated to a small volume, 5 cc. of alumina cream added to precipitate any trace of soil, filtered and washed. The filtrate was evaporated to dryness after adding 1 cc.  $H_2SO_4$  (1:1). The residue was carefully ignited at low red heat to eliminate organic matter and the residue digested in  $HCl$  (1:1) for 2 hours on the steam bath.

TABLE 2  
*Fayette County soil—Total K, 14,000 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		<i>p.p.m.</i>	<i>per cent.*</i>	<i>gm.</i>	<i>per cent.†</i>	<i>gm.</i>	
None	Distilled H <sub>2</sub> O	23	0.16	0.0044	1.92	0.23	Wheat
	0.2 <i>N</i> HNO <sub>3</sub>	107	0.76				
	Sum	130	0.92				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	152	1.09				
S	Distilled H <sub>2</sub> O	29	0.21	0.0076	2.93	0.26	
	0.2 <i>N</i> HNO <sub>3</sub>	103	0.73				
	Sum	132	0.94				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	136	0.97				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	21	0.15	0.0040	1.92	0.21	
	0.2 <i>N</i> HNO <sub>3</sub>	109	0.78				
	Sum	130	0.93				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	107	0.76				

\* Of the total K.

† In air-dry plants.

It was filtered,  $H_2PtCl_6$  added to the filtrate, the latter evaporated and K determined by treatment with acid  $C_2H_5OH$  as described under total K.

*0.2 normal HNO<sub>3</sub>-soluble K.* The soil residue on the filter, obtained in the distilled  $H_2O$  digestion, was allowed to air dry. It was returned to the same bottle and 1100 cc. 0.2*N*  $HNO_3$  added. The soil was digested at room temperature for 5 hours, being shaken at 30-minute intervals, and the solution was then filtered until clear as previously described. An aliquot equivalent to 100 gm. air-dried soil was then concentrated to a small volume and 5 cc. conc.  $HNO_3$  added. This was evaporated to dryness, treatment with  $HNO_3$  was repeated to eliminate organic matter, and finally it was evaporated twice with 5 cc. conc.  $HCl$ . The residue was dried at 120°C. for several hours to dehydrate  $SiO_2$ ,  $HCl$  (1:1) was added and the whole digested on the bath, filtered, and K determined in the same manner previously described.

*NH<sub>4</sub>NO<sub>3</sub>-soluble K.* The digestion was made on the same amount of moist soil in the manner described for the distilled H<sub>2</sub>O extraction. The filtration was made as previously described and an aliquot of the filtrate equivalent to 100 gm. air-dried soil was evaporated to dryness. The bulk of NH<sub>4</sub> salt was eliminated at low heat, 1 cc. H<sub>2</sub>SO<sub>4</sub> (1:1) was then added and the residue

TABLE 3  
*McCracken County soil—Total K, 16,200 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		<i>p.p.m.</i>	<i>per cent</i> *	<i>gm.</i>	<i>per cent</i> †	<i>gm.</i>	
None	Distilled H <sub>2</sub> O	30	0.19	0.0091	2.93	0.31	} Wheat
	0.2 <i>N</i> HNO <sub>3</sub>	90	0.55				
	Sum	120	0.74				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	130	0.80				
S	Distilled H <sub>2</sub> O	45	0.28	0.0087	2.80	0.31	
	0.2 <i>N</i> HNO <sub>3</sub>	81	0.50				
	Sum	126	0.78				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	243	1.50				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	31	0.19	0.0080	3.20	0.25	
	0.2 <i>N</i> HNO <sub>3</sub>	92	0.57				
	Sum	123	0.76				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	178	1.10				
CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	21	0.13	0.0142	1.77	0.80	
	0.2 <i>N</i> HNO <sub>3</sub>	106	0.65				
	Sum	127	0.78				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	93	0.57				
S and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	25	0.15	0.0197	1.89	1.04	} Buck-wheat
	0.2 <i>N</i> HNO <sub>3</sub>	107	0.66				
	Sum	132	0.81				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	95	0.59				
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	22	0.14	0.0091	1.06	0.86	
	0.2 <i>N</i> HNO <sub>3</sub>	102	0.63				
	Sum	124	0.77				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	124	0.77				

\* Of the total K.

† In air-dry plants.

ignited at low red heat to get rid of all NH<sub>4</sub> salt. The residue was taken up with HCl (1:1), digested on a steam bath, filtered, and K determined in the usual manner.

*Sulfates.* Ten grams air-dried soil was shaken with 200 cc. HCl (1 per cent) for 7 hours in a shaking machine, after which the solution was filtered until

clear. An aliquot was concentrated, 2 cc. HCl (1:1) and an excess of hot  $\text{BaCl}_2$  solution (10 per cent) were slowly added. After being heated and standing over night, the  $\text{BaSO}_4$  was filtered, washed, and determined in the usual manner. The precipitate after being weighed was treated with concentrated

TABLE 4  
*Taylor County soil—Total K, 6,500 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		<i>p.p.m.</i>	<i>per cent</i> *	<i>gm.</i>	<i>per cent</i> †	<i>gm.</i>	
None	Distilled H <sub>2</sub> O	47	0.72	0.0118	3.57	0.33	Wheat
	0.2 <i>N</i> HNO <sub>3</sub>	104	1.60				
	Sum	151	2.32				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	162	2.49				
S	Distilled H <sub>2</sub> O	64	0.98	0.0090	2.66	0.34	
	0.2 <i>N</i> HNO <sub>3</sub>	96	1.48				
	Sum	160	2.46				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	252	3.88				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	48	0.74	0.0048	2.18	0.22	
	0.2 <i>N</i> HNO <sub>3</sub>	106	1.63				
	Sum	154	2.37				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	109	1.68				
CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	18	0.28	0.0161	1.55	1.04	
	0.2 <i>N</i> HNO <sub>3</sub>	96	1.48				
	Sum	114	1.76				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	111	1.71				
S‡ and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	23	0.35	0.0147	1.50	0.98	Buck-wheat
	0.2 <i>N</i> HNO <sub>3</sub>	97	1.49				
	Sum	120	1.84				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	121	1.86				
CaSO <sub>4</sub> ·2H <sub>2</sub> O‡ and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	16	0.25	0.0115	1.22	0.94	
	0.2 <i>N</i> HNO <sub>3</sub>	102	1.57				
	Sum	118	1.82				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	104	1.60				

\* Of the total K.

† In air-dry plants.

‡ The amount of S or of  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  added was 500 p.p.m. instead of the usual application of 250 p.p.m.

$\text{H}_2\text{SO}_4$  and HF to eliminate traces of  $\text{SiO}_2$ , and was again weighed. No impurity that would have influenced the result was found to be present.

*Hydrogen-ion concentration.* A colorimetric method was used and the indicators were those recommended by Clark and Lubs (10, p. 66). The procedure

TABLE 5  
*Shelby County soil—Total K, 32,600 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		<i>p.p.m.</i>	<i>per cent</i> *	<i>gm.</i>	<i>per cent</i> †	<i>gm.</i>	
None	Distilled H <sub>2</sub> O	19	0.06	0.0074	2.38	0.31	Wheat
	0.2 <i>N</i> HNO <sub>3</sub>	136	0.42				
	Sum	155	0.48				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	217	0.67				
S	Distilled H <sub>2</sub> O	28	0.09	0.0048	2.08	0.23	
	0.2 <i>N</i> HNO <sub>3</sub>	134	0.41				
	Sum	162	0.50				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	226	0.69				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	20	0.06	0.0069	2.31	0.30	
	0.2 <i>N</i> HNO <sub>3</sub>	133	0.41				
	Sum	153	0.47				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	188	0.58				

\* Of the total K.

† In air-dry plants.

TABLE 6  
*Franklin County soil—Total K, 35,100 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		<i>p.p.m.</i>	<i>per cent</i> *	<i>gm.</i>	<i>per cent</i> †	<i>gm.</i>	
None	Distilled H <sub>2</sub> O	19	0.05	0.0058	2.15	0.27	Wheat
	0.2 <i>N</i> HNO <sub>3</sub>	161	0.46				
	Sum	180	0.51				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	192	0.55				
S	Distilled H <sub>2</sub> O	24	0.07	0.0100	2.87	0.35	
	0.2 <i>N</i> HNO <sub>3</sub>	159	0.45				
	Sum	183	0.52				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	203	0.58				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	18	0.05	0.0104	2.67	0.39	
	0.2 <i>N</i> HNO <sub>3</sub>	148	0.42				
	Sum	166	0.47				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	185	0.53				

\* Of the total K.

† In air-dry plants.

was to take 0.2 gm. of air-dried soil previously ground in a mortar and moisten it on an unglazed porcelain plate with 4 or 5 drops of aqueous indicator, sufficient being added so that the soil was saturated and the liquid had a tend-

ency to leave the soil when the plate was inclined. After 1 minute the liquid was drawn away from the soil with the point of a knife, spread, and the color compared with the Clark and Lubs standard color chart (10, p. 41). This method probably would be expected to give only comparative results but no difficulty was found in duplicating with different indicators where they could be used. Moreover it shows large differences in the same soil, with and without the addition of  $\text{CaCO}_3$ , and for this purpose mainly it was used.

*K in plant ash.* LeClerc and Breazeale (15) have shown that young wheat seedlings for a few weeks after sprouting have a great avidity for extracting K from culture solutions. It was thought that possibly, because of occasion by the soil, the K liberated by the treatments might not be extracted by the solvent and yet might be utilized by these plants. Therefore, their procedure

TABLE 7  
*Madison County soil No. 1—Total K, 29,100 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	per cent*	gm.	per cent†	gm.	
None	Distilled H <sub>2</sub> O	21	0.07	0.0067	2.30	0.29	Wheat
	0.2 <i>N</i> HNO <sub>3</sub>	195	0.67				
	Sum	216	0.74				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	380	1.51				
S	Distilled H <sub>2</sub> O	24	0.08	0.0161	3.84	0.42	
	0.2 <i>N</i> HNO <sub>3</sub>	186	0.64				
	Sum	210	0.72				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	240	0.82				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	33	0.11	0.0086	2.68	0.32	
	0.2 <i>N</i> HNO <sub>3</sub>	176	0.60				
	Sum	209	0.71				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	587	2.02				

\* Of the total K.

† In air-dry plants.

modified to suit soil conditions was tried and wheat seedlings were grown in some of the treated soils for 2 weeks. Similar experiments were made on others with buckwheat seedlings which were grown for 3 weeks. The latter plants have been reported by Haley (13) as also having an avidity for K in culture experiments.

In each case 100 germinated seeds were planted in 100 gm. of air-dried soil in a shallow dish. The seeds were previously germinated so as to insure their viability and uniformity in number.

The soils for these experiments were allowed to air-dry, the germinated seed were planted and a uniform moisture content was afterwards maintained. At the end, the plants were cut close to the ground, washed with distilled



H<sub>2</sub>O, air-dried, and weighed. The entire lot from each treatment was moistened with concentrated H<sub>2</sub>SO<sub>4</sub> and ashed until the organic matter was destroyed. To the residue, HCl (1:1) was added, the whole was digested on the steam bath and filtered. The filtrate was evaporated with H<sub>2</sub>PtCl<sub>6</sub>, the residue treated with acid C<sub>2</sub>H<sub>5</sub>OH, and K determined. These experiments were carried on at the same time as the digestions.

TABLE 8  
*Madison County soil No. 2—Total K, 9,500 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		<i>p.p.m.</i>	<i>per cent</i> *	<i>gm.</i>	<i>per cent</i> †	<i>gm.</i>	
None	Distilled H <sub>2</sub> O	97	1.02	0.0227	2.32	0.98	Buck- wheat
	0.2 <i>N</i> HNO <sub>3</sub>	123	1.29				
	Sum	220	2.31				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	255	2.68				
S	Distilled H <sub>2</sub> O	104	1.09	0.0208	2.17	0.96	
	0.2 <i>N</i> HNO <sub>3</sub>	116	1.22				
	Sum	220	2.31				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	202	2.13				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	101	1.06	0.0081	0.97	0.84	
	0.2 <i>N</i> HNO <sub>3</sub>	125	1.32				
	Sum	226	2.38				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	200	2.11				
CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	61	0.64	0.0120	1.35	0.89	
	0.2 <i>N</i> HNO <sub>3</sub>	140	1.47				
	Sum	201	2.11				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	167	1.76				
S and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	66	0.69	0.0139	1.60	0.87	
	0.2 <i>N</i> HNO <sub>3</sub>	132	1.39				
	Sum	198	2.08				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	163	1.72				
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	59	0.62	0.0017	1.09	0.16	
	0.2 <i>N</i> HNO <sub>3</sub>	141	1.48				
	Sum	200	2.10				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	174	1.83				

\* Of the total K.

† In air-dry plants.

The results of the solubility tests and plant studies are given in tables 1 to 14 and the other determinations in table 15. In reporting gains or losses, only those amounting to over 5 per cent were considered.

## DISCUSSION OF RESULTS AND CONCLUSIONS

This investigation consists mainly of solubility tests of K in 11 Kentucky soils of different types which were subjected to different treatments. The soils were mixed with S or  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  and in some instances,  $\text{CaCO}_3$  was added

TABLE 9  
*Muhlenburg County soil—Total K, 12,300 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p. p. m.	per cent*	gm.	per cent†	gm.	
None	Distilled H <sub>2</sub> O	22	0.18	0.0202	2.06	0.98	Buck-wheat
	0.2 <i>N</i> HNO <sub>3</sub>	73	0.59				
	Sum	95	0.77				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	86	0.70				
S	Distilled H <sub>2</sub> O	27	0.22	0.0289	2.60	1.11	
	0.2 <i>N</i> HNO <sub>3</sub>	75	0.61				
	Sum	102	0.83				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	77	0.63				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	22	0.18	0.0131	1.47	0.89	
	0.2 <i>N</i> HNO <sub>3</sub>	77	0.63				
	Sum	99	0.81				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	75	0.61				
CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	17	0.14	0.0130	1.48	0.88	
	0.2 <i>N</i> HNO <sub>3</sub>	77	0.63				
	Sum	94	0.77				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	88	0.72				
S and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	16	0.13	0.0078	0.95	0.82	
	0.2 <i>N</i> HNO <sub>3</sub>	77	0.63				
	Sum	93	0.76				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	70	0.57				
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	16	0.12	0.0112	1.29	0.87	
	0.2 <i>N</i> HNO <sub>3</sub>	80	0.65				
	Sum	96	0.77				
	0.1 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	84	0.68				

\* Of the total K.

† In air-dry plants.

together with either of the above materials. The treated soils were maintained at a uniform moisture content for 4 months, at the temperature of the laboratory, and were frequently stirred. The soluble K was then determined by digesting them in different solvents (distilled  $\text{H}_2\text{O}$ , 0.2N  $\text{HNO}_3$  and either 0.1M or 0.2M  $\text{NH}_4\text{NO}_3$ ).

As young wheat and buckwheat plants have been reported to have an avidity for soluble K, they were grown in the treated soils for a short time at the end of the 4-month period of digestion and the K in the plant was determined. The plants grew well in all cases, except that on one treated soil of Madison County, No. 2, the buckwheat did not do so well as on the other

TABLE 10  
*Logan County soil—Total K, 14,600 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		<i>p.p.m.</i>	<i>per cent</i> *	<i>gm.</i>	<i>per cent</i> †	<i>gm.</i>	
None	Distilled H <sub>2</sub> O	14	0.10	0.0094	1.07	0.88	Buck- wheat
	0.2 <i>N</i> HNO <sub>3</sub>	67	0.46				
	Sum	81	0.56				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	68	0.47				
S	Distilled H <sub>2</sub> O	18	0.12	0.0149	1.60	0.93	
	0.2 <i>N</i> HNO <sub>3</sub>	65	0.45				
	Sum	83	0.57				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	66	0.45				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	14	0.10	0.0151	1.51	1.00	
	0.2 <i>N</i> HNO <sub>3</sub>	67	0.46				
	Sum	81	0.56				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	57	0.39				
CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	10	0.07	0.0077	0.90	0.85	
	0.2 <i>N</i> HNO <sub>3</sub>	80	0.55				
	Sum	90	0.62				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	59	0.40				
S and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	13	0.09	0.0141	1.48	0.95	
	0.2 <i>N</i> HNO <sub>3</sub>	76	0.52				
	Sum	89	0.61				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	67	0.46				
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	9	0.06	0.0103	1.14	0.90	
	0.2 <i>N</i> HNO <sub>3</sub>	74	0.51				
	Sum	83	0.57				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	99	0.68				

\* Of the total K.

† In air-dry plants.

samples of this soil. These experiments were used as a further test for liberated K, since it was thought that possibly some of the liberated K might be adsorbed by the soil and hence would not be obtained in solution but nevertheless might be utilized by the plant.

Table 12 shows that all soils gave increases in  $H_2O$ -soluble K with the S treatment and 5 out of 7 tested show gains even when  $CaCO_3$  was added with S. Only one soil, however, produced more  $H_2O$ -soluble K with  $CaSO_4 \cdot 2H_2O$ , although 3 soils with this material gave an increased amount of K in the  $NH_4NO_3$  digestion.

TABLE 11  
*Laurel County soil—Total K, 12,200 p.p.m.*

ADDITIONS	SOLVENTS USED	POTASSIUM (K) EXTRACTED				WEIGHT OF THE AIR-DRY PLANTS	PLANT GROWN
		By solvents		By plants			
		p.p.m.	per cent*	gm.	per cent†	gm.	
None	Distilled H <sub>2</sub> O	49	0.40	0.0092	1.12	0.82	Buck-wheat
	0.2 <i>N</i> HNO <sub>3</sub>	119	0.98				
	Sum	168	1.38				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	162	1.33				
S	Disilled H <sub>2</sub> O	57	0.470	0.0099	1.19	0.83	
	0.2 <i>N</i> HNO <sub>3</sub>	111	0.91				
	Sum	168	1.38				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	156	1.28				
CaSO <sub>4</sub> ·2H <sub>2</sub> O	Distilled H <sub>2</sub> O	49	0.40	0.093	1.05	0.89	
	0.2 <i>N</i> HNO <sub>3</sub>	117	0.96				
	Sum	166	1.36				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	147	1.20				
CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	29	0.24	0.0150	1.67	0.90	
	0.2 <i>N</i> HNO <sub>3</sub>	125	1.02				
	Sum	154	1.26				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	119	0.98				
S and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	55	0.45	0.0173	1.99	0.87	
	0.2 <i>N</i> HNO <sub>3</sub>	105	0.86				
	Sum	160	1.31				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	127	1.04				
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	Distilled H <sub>2</sub> O	28	0.23	0.0134	1.46	0.92	
	0.2 <i>N</i> HNO <sub>3</sub>	126	1.03				
	Sum	154	1.26				
	0.2 <i>M</i> NH <sub>4</sub> NO <sub>3</sub>	124	1.02				

\* Of the total K.

† In air-dry plants.

According to table 7, a greater quantity of K was found in wheat grown in Madison No. 1 soil after the  $CaSO_4 \cdot 2H_2O$  treatment, and larger amounts of the same element were extracted by  $H_2O$  and  $NH_4NO_3$ . This indicates that  $CaSO_4 \cdot 2H_2O$  may have liberated K in this soil. The results on the other soils, however, generally were not so good with  $CaSO_4 \cdot 2H_2O$  as with S.

TABLE 12  
*Effect of treatments on soluble potassium in soils (tables 1 to 11)*

ADDITIONS	NUMBER OF SOILS	
<i>H<sub>2</sub>O-soluble K</i>		
S.....	11	Gain in all, particularly 4 (McCracken, Taylor, Shelby, Laurel)
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	11	Gain in 1 (Madison No. 1); loss in 1 (Fayette)
CaCO <sub>3</sub> .....	7	No gain; loss in all, particularly 5 (Graves, McCracken, Taylor, Madison No. 2, Laurel)
S and CaCO <sub>3</sub> *.....	7	Gain in 5 (McCracken, Taylor, Madison No. 2, Logan, Laurel). Loss in 1 (Muhlenburg).
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub> *.....	7	No gain; loss in 4 (Graves, Taylor, Muhlenburg, Logan).
<i>0.2 normal HNO<sub>3</sub>-soluble K</i>		
S.....	11	No gain; loss in 5 (Graves, McCracken, Taylor, Madison No. 2, Laurel)
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	11	No gain; loss in 3 (Graves, Franklin, Madison No. 1)
CaCO <sub>3</sub> .....	7	Gain in 3 (McCracken, Madison No. 2, Logan); loss in 2, (Graves, Taylor).
S and CaCO <sub>3</sub> *.....	7	No gain; loss in 2 (Madison No 2 and Laurel).
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub> *.....	7	Gain in 1 (Taylor); loss in 1 (Logan).
<i>H<sub>2</sub>O-soluble K + 0.2 normal HNO<sub>3</sub>-soluble K</i>		
S.....	11	Gain in 2 (Taylor, Muhlenburg)
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	11	No gain; loss in 1 (Franklin)
CaCO <sub>3</sub> .....	7	Gain in 2 (McCracken, Logan); loss in others except Muhlenburg.
S and CaCO <sub>3</sub> *.....	7	No gain or loss
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub> *.....	7	No gain; loss in 1 (Logan)
<i>NH<sub>4</sub>NO<sub>3</sub>-soluble K</i>		
S.....	11	Gain in 4 (Graves, McCracken, Taylor, Franklin); loss in 4 (Fayette, Madison Nos. 1 and 2, Muhlenburg)
CaSO <sub>4</sub> ·2H <sub>2</sub> O.....	11	Gain in 3 (Graves, McCracken, Madison No. 1); loss in remainder except Franklin.
CaCO <sub>3</sub> .....	7	No gain; loss in all except Muhlenburg.
S and CaCO <sub>3</sub> *.....	7	Gain in 3 (Taylor, Logan, Laurel); loss in 1 (Muhlenburg)
CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub> *.....	7	Gain in 2 (McCracken, Logan); loss in 1. (Taylor).

\* Combined treatment compared with CaCO<sub>3</sub>.

There appears to be no consistent correlation between the amounts of K extracted by the solvent and by the plant except in a few instances. Table 14 shows, however, that those soils which gave an increase of K in the plant, in the majority of instances also show an increase in  $H_2O$ -soluble K. As for the other solvents in this table, usually the contrary was true although more agreement with the plant is shown by  $NH_4NO_3$  than by  $HNO_3$ . This indicates that the plants obtained the K for their initial growth from that which was soluble rather than absorbed.

TABLE 13  
*Effect of treatments on potassium in plants grown in treated soils (tables 1 to 11)*

ADDITIONS	NUMBER OF SOILS	
<i>Wheat</i>		
S.....	7	Gain in 3 (Fayette, Franklin, Madison No. 1) both in per cent and weight; loss in others except McCracken.
$CaSO_4 \cdot 2H_2O$ .....	7	Gain in 3 (McCracken, Franklin, Madison No. 1) in per cent but only in last 2 in weight. Loss in remainder either in per cent or weight.
<i>Buckwheat</i>		
S.....	4	Gain in 3 (Muhlenburg, Logan, Laurel) both in per cent and weight; loss in 1 (Madison No. 2).
$CaSO_4 \cdot 2H_2O$ ....	4	Gain in 1 (Logan) both in per cent and weight; loss in remainder either in per cent or weight.
$CaCO_3$ .....	7*	Gain in 1 (Laurel) both in per cent and weight; loss in remainder.
S and $CaCO_3$ †.....	7	Gain in 4 (McCracken, Madison No. 2, Logan, Laurel) both in per cent and weight; loss in 2 (Taylor, Muhlenburg) either in per cent or weight.
$CaSO_4 \cdot 2H_2O$ and $CaCO_3$ †.....	7	Gain in 1 (Logan) both in per cent and weight; loss in remainder.

\* There were only 4 samples in which this comparison could be made because wheat was grown in the other 3 untreated soils.

† Combined treatment compared with  $CaCO_3$ .

The  $CaCO_3$  generally had a beneficial effect on the oxidation of S but a depressive one on the soluble K extracted by every solvent except 0.2*N*  $HNO_3$ . For instance, in table 15, according to the added S oxidized, the combination of S and  $CaCO_3$  showed gains over S in 6 soils, varying from 16 per cent in Laurel County to 3862 per cent in Taylor County. The Logan County sample was the only exception and this showed a loss of 6 per cent. On the other hand, table 12 shows that  $CaCO_3$  depressed the solubility of K in distilled  $H_2O$  in every soil and the solubility in  $NH_4NO_3$ -solution in all except

Muhlenburg County. This is of interest inasmuch as it shows that  $\text{CaCO}_3$  apparently has contradictory functions, one beneficial, to promote the oxida-

TABLE 14

*Correlation of gains in potassium shown by plants and solubility tests in different treatments of soils (tables 12-13)\**

soil†	WHEAT	H <sub>2</sub> O-SOLUBLE K	0.2 N HNO <sub>3</sub> -SOLUBLE K	H <sub>2</sub> O-SOLUBLE K + 0.2 N HNO <sub>3</sub> -SOLUBLE K	NH <sub>4</sub> NO <sub>3</sub> -SOLUBLE K
<i>S</i>					
Fayette.....	+	+			+
Franklin.....	+	+			
Madison No. 1.....	+	+			
<i>CaSO<sub>4</sub>·2H<sub>2</sub>O</i>					
McCracken.....	+‡				+
Franklin.....	+				
Madison No. 1.....	+	+			+
<i>S</i>					
	BUCKWHEAT				
Muhlenburg.....	+	+		+	
Logan.....	+	+			
Laurel.....	+	+			
<i>CaSO<sub>4</sub>·2H<sub>2</sub>O</i>					
Logan.....	+				
<i>CaCO<sub>3</sub></i>					
Laurel.....	+		+		
<i>S and CaCO<sub>3</sub>§</i>					
McCracken.....	+	+			
Madison No. 2.....	+	+			
Logan.....	+	+			+
Laurel.....	+	+			+
<i>CaSO<sub>4</sub>·2H<sub>2</sub>O and CaCO<sub>3</sub>§</i>					
Logan.....	+				+

\* Gains indicated by +. Blank spaces indicate no gain and frequently a loss.

† Only the soils are mentioned in which the plant grown in the treated soil showed a gain both in per cent and weight of potassium.

‡ Only in per cent of potassium.

§ Combined treatment compared with  $\text{CaCO}_3$ .

tion of  $\text{S}$  to  $\text{H}_2\text{SO}_4$  which reacts with the soil silicates to form soluble K, and the other detrimental since it seems to increase the adsorption of K by the soil.

Its detrimental effect might be partly explained as due to its neutralization of the acid before the latter can act on the soil, but nevertheless a comparison

TABLE 15  
*Sulfur oxidized and hydrogen-ion concentration at the end of the experiments*

SOIL	ADDITIONS	SULFATE S		ADDED S OXIDIZED		pH
		Initial amount	Final amount	Amount	Per cent of amount added	
		<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>		
Graves County	None	46	121	—	—	6.5
	S	—	197	76	30	6.3
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.5
	CaCO <sub>3</sub>	—	73	—	—	8.5
	S and CaCO <sub>3</sub>	—	250	177	71	8.4
	CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	—	—	—	—	8.6
Fayette County	None	101	155	—	—	6.3
	S	—	191	36	14	6.3
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.3
McCracken County	None	43	41	—	—	6.7
	S	—	75	34	14	6.5
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.6
	CaCO <sub>3</sub>	—	69	—	—	8.4
	S and CaCO <sub>3</sub>	—	269	200	80	8.3
	CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	—	—	—	—	8.3
Taylor County	None	40	87	—	—	6.3
	S	—	95	8	3	6.3
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.3
	CaCO <sub>3</sub>	—	35	—	—	8.4
	S* and CaCO <sub>3</sub>	—	360	325	65	8.2
	CaSO <sub>4</sub> ·2H <sub>2</sub> O* and CaCO <sub>3</sub>	—	—	—	—	8.3
Shelby County	None	40	67	—	—	6.1
	S	—	78	11	4	6.1
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.1
Franklin County	None	53	66	—	—	6.9
	S	—	69	3	1	6.8
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.9
Madison County No. 1	None	136	91	—	—	7.0
	S	—	111	20	8	6.9
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	7.0

\* The amounts of S and CaSO<sub>4</sub>·2H<sub>2</sub>O added were 500 p.p.m. instead of the usual applications of 250 p.p.m.



TABLE 15—*Continued*

SOIL	ADDITIONS	SULFATE S		ADDED S OXIDIZED		pH
		Initial amount	Final amount	Amount	Per cent of amount added	
		<i>p. p. m.</i>	<i>p. p. m.</i>	<i>p. p. m.</i>		
Madison County No. 2	None	78	104	—	—	6.1
	S	—	253	149	60	6.0
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.1
	CaCO <sub>3</sub>	—	115	—	—	8.2
	S and CaCO <sub>3</sub>	—	308	193	77	8.2
	CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	—	—	—	—	8.2
Muhlenburg County	None	55	78	—	—	6.4
	S	—	185	107	43	6.1
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.2
	CaCO <sub>3</sub>	—	43	—	—	8.3
	S and CaCO <sub>3</sub>	—	188	145	58	8.1
	CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	—	—	—	—	8.2
Logan County	None	47	61	—	—	6.3
	S	—	234	173	69	6.0
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.3
	CaCO <sub>3</sub>	—	66	—	—	8.5
	S and CaCO <sub>3</sub>	—	229	163	65	8.2
	CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	—	—	—	—	8.4
Laurel County	None	41	44	—	—	6.3
	S	—	156	112	45	6.2
	CaSO <sub>4</sub> ·2H <sub>2</sub> O	—	—	—	—	6.2
	CaCO <sub>3</sub>	—	18	—	—	8.4
	S and CaCO <sub>3</sub>	—	148	130	52	8.2
	CaSO <sub>4</sub> ·2H <sub>2</sub> O and CaCO <sub>3</sub>	—	—	—	—	8.2

of the combined S and CaCO<sub>3</sub> treatments with the corresponding untreated soils seems to show that the base has increased the capacity of the soil to adsorb the soluble K as shown both in the H<sub>2</sub>O and NH<sub>4</sub>NO<sub>3</sub> digestions.

In the majority of treatments without CaCO<sub>3</sub>, the amount of K soluble in NH<sub>4</sub>NO<sub>3</sub> solution was larger than the combined amount obtained by distilled H<sub>2</sub>O and 0.2*N* HNO<sub>3</sub>, whereas with this base present, it was less. Moreover, with the same solvent, when CaCO<sub>3</sub> was present in the treatment, the K obtained was generally less than that extracted from the untreated soil.

There was very little variation in the hydrogen-ion concentration in the different treatments of the same soil, according to the method used, except when CaCO<sub>3</sub> was added. The amounts of S oxidized in some soils indicate that considerable quantities of acid were produced.

The fact that all samples to which  $\text{CaCO}_3$  was added were rendered alkaline and all except the Logan County sample oxidized larger quantities of S, indicates that the S organisms to which an alkaline medium is favorable, were predominant in these soils.

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# EFFECT OF GROWING LEGUMES UPON SUCCEEDING CROPS

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## LITERATURE

It has been known since ancient times that legumes exert a beneficial effect upon the succeeding crops. This knowledge has played an important part in the development of crop rotations in Europe, and early American authors (3, 28) have likewise emphasized the good after-effect of clover. But how this beneficial effect is being accomplished has not been fully explained.

There are several causes that have to be considered: (a) an increase in the soil nitrogen by the crop residues, (b) an increase of potash and phosphate in the surface soil due to the transportation of these substances from the subsoil by deep-rooted legumes, (c) a change in soil reaction, (d) an improved physical structure of the soil, (e) suppression of weeds, (f) increased bacterial activities.

Naturally, most attention has been paid to the increase in soil nitrogen. Long continued experiments made at the Rothamsted Experiment Station have shown very conspicuous accumulations of nitrogen under legumes (7), and numerous other tests have given similar results. On the other hand, several authors have strongly emphasized, that if only stubble and roots are left on the field no increase in nitrogen takes place (9, 16, 27, 34, 35, 36). Investigations made at the Wyoming Station (4) have demonstrated that alfalfa cut for hay exerted its characteristic beneficial after effect upon wheat and oats although the roots were free from nodules, and therefore no symbiotic nitrogen fixation could have taken place. Therefore, it is very probable that wherever an increase becomes noticeable in the soil nitrogen under and after legumes, this is much less due to the nitrogen fixation by the bacteria in the root nodules, than to the action of non-symbiotic soil organisms assimilating elementary nitrogen.

The lifting of potash and phosphate from the subsoil by deep rooted legumes is another point upon which not much accurate information is available. At the Kansas Experiment Station uninoculated soybeans that were free from nodules, increased the following wheat crop very distinctly. The investigators were of the opinion that this effect was partly due to the potash and phosphate liberated from the decaying roots (5). More recently this point was emphasized in explaining results obtained in Germany with stubble and roots of lupines (10).

The increase in soil acidity to which several authors have ascribed occasional unsatisfactory effects of green manuring (30, 31), is probably of very little importance in this case (17) as well as the after-effect of harvested legumes. The crop rotation experiments carried on at the Rhode Island Station have shown that in certain cases the acidity factor may be of considerable importance (13), but hardly anything is known about the behavior of the different legumes. A few data obtained at the Cornell Station have been explained as

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indicating an increased soil acidity after harvested legumes (25). The beneficial after-effect of legume growth is not reduced, however, by liming, as will be shown presently. Therefore, increased soil acidity can not be of considerable importance in this case.

The possibility of improvement of physical soil structure under a heavy growth of legumes, which has always attracted the attention of careful observers (12, 14, 28), deserves the full attention of the practical farmer, at least in regions with insufficient rainfall. The growing crop of legumes needs much water; therefore, if rain is lacking this beneficial effect can not be expected, and fallowing will be preferable.

The suppression of weeds by the growing legumes is another point of great practical importance. Naturally, this benefit will be fully realized only if a close, heavy development of legumes is secured. Therefore, accurate knowledge of the kind of legumes best suited to the particular soil and climate is again much to be desired. The water saved for the succeeding crop by the suppression of weeds is undoubtedly in many cases an important, though frequently overlooked, contributing cause to the beneficial influence exerted by the preceding legumes.

Increased bacterial action, especially intensified nitrification under legumes, also deserves careful investigation (23, p. 767). As early as in 1895 the nitrate accumulation under legumes was strongly emphasized by Laws and Gilbert (18, p. 130). Experiments made at Rothamsted in 1885, which were published in 1909 (7) showed nitrate present in large amounts under white and sweet clovers, whereas it was almost absent under alfalfa. Lyon and his associates noticed high nitrification under alfalfa and clover, whereas it was low under soybeans (24, 25). LeClair (19) found strong nitrifying efficiency under cowpeas, and Holtz and Singleton (15) again under alfalfa and sweet clover.

Conspicuous increases in the total number of soil bacteria and in the carbon dioxide produced by them have also been observed repeatedly. Two-to-four-fold increases have been recorded in experiments with cowpeas (19). According to Stoklasa (33) higher counts and stronger CO<sub>2</sub> production are to be expected under alfalfa and red clover than under cereals.

That especially non-symbiotic nitrogen fixing bacteria are stimulated in their development by the legumes was pointed out by Liebscher and by Beijerinck about 20 and 30 years ago (23, p. 770), but nothing more definite has become known since then. Field experiments made in West Virginia (2) gave apparent gains in nitrogen of 78 pounds per acre and year, when in a 16-year rotation cowpeas or clover were grown four times and phosphate and potash were applied as fertilizers. Since the surface growth of the legumes was removed, the results obtained would constitute a definite proof of strong nitrogen fixation in the soil, were it not for the uncertainty of soil nitrogen determinations.

#### EXPERIMENTAL RESULTS

A combination of field, greenhouse, and laboratory experiments, was adopted to secure more complete information on the effect of growing legumes upon succeeding crops. The results of field experiments are of greatest practical value, but considerable uncertainty is frequently attached to them. Experiments in the greenhouse allow a much better control of all conditions but as these conditions and the results are more or less different from those obtainable in the field, the underlying causes of the results are often left in doubt. Suitably arranged laboratory experiments are therefore necessary. A careful co-ordination of the different lines of work can do much to enhance the advantages and to reduce the disadvantages of the three ways of investigating problems in soil bacteriology.

*Field experiments*

An area of about three acres at Arlington Experimental Farm, that had been kept permanently under grass, was selected in 1914 and laid out into 120 plots of slightly varying size. Field I consisted of 48 plots, each  $\frac{1}{8}$  acre. Three sets of 24 plots each, wherein every plot was  $\frac{1}{8}$  acre, were designated fields II A, II B, and III. The grass was cut twice on the marked plots in 1914, and after the weights obtained had demonstrated a fair uniformity of the soil, the permanent field experiments were started in the fall. In order to secure definite information upon the suitability of the land, no fertilizer or manure was applied to the 1915 crop.

Chemical and biological tests indicated that the rather heavy clay soil of the field, contains much potash (1.4 per cent  $K_2O$ ) and phosphate (0.25 per cent  $P_2O_5$ ). Neither potash nor phosphate, nor both combined, either with or without mineral or organic nitrogen, exerted any effect upon the crops grown on small test plots at the ends of the fields.

The original soil reaction was pH 6.4, but in view of the physical structure of the soil the whole area received an application of slaked lime before the sod was broken and this treatment was repeated regularly as noted below ( $1\frac{1}{2}$  tons per acre for an eight-year rotation).

The soil of field I is somewhat lighter than that of fields II and III. The original nitrogen content was 0.07 per cent on field I, and 0.10 per cent on fields II and III.

The crop rotations, as planned originally, were as follows:

*Field I*

1. Wheat [Hairy Vetch and Rye]
2. Soybeans and Cowpeas, or Beets
3. Corn
4. Potatoes +
5. Oats [Hairy Vetch and Rye]
6. Soybeans and Cowpeas, or Beets
7. Corn
8. Potatoes +

*Fields II and III*

1. Wheat [Cowpeas, Soybeans]
2. Potatoes +
3. Wheat [Cowpeas, Soybeans]
4. Corn
5. Corn +
6. Oats [Clover and Grass]
7. Clover and Grass
8. Clover and Grass

The main difference between the rotations is in the crop succession. On field I the grain crops are separated from each other by legumes or potatoes, as is customary in Europe; on fields II and III the grain crops follow each other, as is frequently practised in America. One-year wheat had to be replaced by another year of potatoes on field I, and instead of clover, soybeans and cowpeas were grown. The catch crops after wheat, given in brackets, were likewise different. The crops receiving stable manure are marked +. Lime was regularly applied to corn on field I, and to oats on fields II and III. On four plots of field I the legumes were replaced by beets.

Since the topography of the area did not permit a further extension of the experimental plots on field I, only four crops could be grown simultaneously, whereas on fields II and III the complete rotation was repeated each year. On account of the uniform distribution of the various crops in the first rotation,

TABLE 1  
*Nitrogen (pounds per acre) in crops harvested 1915 to 1924\**  
 All (main) crops = averages of 6 years of cereals and potatoes, 2 years of legumes, beets, or clover and grass

YIELD	TREATMENT	CROPS	1915	1916	1917	1920	1921	1922	1923	1924	8 YEARS TOTAL
I	No nitrogen	Cereals and potatoes	20.42	32.20	28.56	23.61	20.15	14.61	39.34	32.91	211.80
		Beets	45.50	78.62	29.64	21.74	Failed	67.08	37.96	31.32	311.86
		All crops	27.08	43.63	28.87	23.21	15.00	28.50	39.05	34.51	239.85
	Mineral nitrogen	Cereals and potatoes	18.81†	38.34	57.60	36.83	21.36†	19.40	46.80	44.19	283.33
		Beets	42.38†	108.76	52.91	19.11	Failed	77.70	57.33	37.80	395.99
		All crops	24.91†	56.41	56.49	32.35	15.90†	34.47	49.61	42.28	313.42
	Legumes	Cereals and potatoes	19.71	36.98	42.93	38.26	22.86	29.97	47.25	41.40	279.36
		Legumes	55.38	150.54	105.56	52.39	102.60	72.90	86.21	66.15	691.73
		Main crops	27.62	66.38	59.14	41.25	42.40	40.36	56.70	47.17	381.02
	Legumes and fresh stable manure	Catch crops	.....	43.16	53.04	76.28	57.78	32.94	47.79	....†	310.99
		Cereals and potatoes	18.38†	41.22	49.95	41.70	28.44	30.51	67.89	50.58	328.67
		Legumes	61.56	138.08	132.35	54.65	128.09	84.08	93.61	73.17	765.59
		Main crops	28.96†	64.95	70.03	44.60	52.94	43.58	73.77	55.74	434.57
		Catch crops	.....	41.18	58.24	68.85	60.62	54.22	71.25	....†	354.36

I	Legumes and 5 weeks old stable manure	Cereals and potatoes Legumes	20.43† 57.05	40.41 154.31	53.01 134.87	41.65 53.51	26.91 130.06	28.45 103.00	66.25 86.59	48.06 75.87	325.17 795.26
		Main crops									
		Catch crops	.....	35.02	67.01	64.13	58.56	53.87	70.71	.....†	349.30
	Legumes and 10 weeks old stable manure	Cereals and potatoes Legumes	19.80† 63.35	42.30 153.23	55.53 124.39	47.62 56.65	26.59 130.84	27.58 108.60	74.18 78.65	50.85 72.90	344.45 788.61
		Main crops	30.40†	69.50	71.96	49.51	52.26	47.47	75.48	56.01	452.59
		Catch crops	.....	36.26	67.72	68.18	59.76	55.40	70.28	.....†	357.60
	No nitrogen	Cereals and potatoes Clover and grass	43.93 36.90	51.73 130.16	49.21 163.60	28.73 45.59	25.71 33.82	32.42 13.17	50.84 8.00	47.00 58.70	329.57 489.94
		All crops	42.18	70.85	77.81	32.45	27.74	27.61	40.13	49.92	368.69
	Mineral nitrogen	Cereals and potatoes Clover and grass	44.73† 33.20†	62.23 143.91	56.83 172.36	33.70 46.65	29.45 34.87	31.66† 11.09†	51.72 9.00	53.73 56.11	364.05 807.19
		All crops	41.85†	82.65	85.71	36.94	30.80	24.52†	41.04	55.07	398.58
	Legumes	Cereals and potatoes Clover and grass	41.07 37.80	55.26 100.46	50.63 161.58	37.46 44.49	28.81 35.72	30.81 12.28	46.01 8.90	45.43 65.13	335.48 466.36
		Main crops	40.25	66.56	78.37	41.72	30.54	26.18	36.74	50.36	370.72
		Catch crops	43.00	47.92	Failures	Failures	Failures	Failures	131.40	65.60	287.92

\* Exclusive of crops of 1918 and 1919.

† No mineral nitrogen and no stable manure in 1915; no mineral nitrogen on field I in 1921, on field II/III in 1922; no catch crop on field I in 1924 because of change in rotation.



this arrangement could be made without endangering the reliability of the results obtained.

The differential treatment on field I was as follows:

Two strips running across the four differently planted strips received no nitrogenous manure, and two received nitrate and ammonium sulfate equivalent to an average application of 30 pounds nitrogen per acre and year (240 pounds for the full rotation). On these four strips beets were planted instead of legumes. On the remaining eight strips, legumes were grown as source of nitrogen and six of them received in addition, a small application of cow manure (equal to 15 tons containing approximately 160 pounds nitrogen per acre, for the eight-year rotation). This manure was spread on two strips approximately 10 weeks before planting; on two, about 5 weeks later; and on the remaining two strips, immediately before they were prepared for the planting of potatoes.

Fields II A, II B, and III were divided in thirds across the full rotation. Clover was grown on all plots, and stable manure (in this case horse manure in the same quantity as above) was applied to all plots. One crosswise strip received no further treatment; on another one, nitrate and ammonium sulfate were applied as on field I; and on the third strip, catch crops were grown after wheat. After cowpeas and soybeans had failed repeatedly (in 1917 and 1920) as catch crops sweet clover was tried (in 1921 and 1922), but it likewise proved unsatisfactory. Therefore, hairy vetch mixed with rye was adopted in this case (in 1923 and 1924).

The plan as developed for field I made it possible to observe how mineral nitrogenous fertilizers, the growth of legumes, and the earlier or later application of stable manure would increase the crops above those grown without manuring. On fields II and III on the other hand, all plots were under the influence of clover growth and the application of horse manure, and it remained to be seen what further increases could be secured by the additional use of mineral nitrogen or by the growth of catch crops.

The clover was regularly harvested on all plots, but the plots of fields II and III bearing catch crops, and all plots planted to legumes on field I were equally divided in each case. On one-half of each plot the surface growth was removed, on the other half it was plowed under.

As these experiments had to be stopped temporarily during the war period, all plots that should not get the benefit of leguminous crops were laid down to grass, and on the other plots a mixture of cowpeas and soybeans was sown each year and all growth was removed as hay. Accordingly, no crops are recorded for 1918 and 1919 in table 1. With the exception of the legume strips, the crops harvested in 1920 were as low as, or lower than, those of 1915, especially on fields II and III.

As the first crop in 1915 received neither stable manure nor mineral nitrogen, and as the mineral nitrogen was omitted on field I in 1921 and on fields II and III in 1922, the total nitrogen applied to the full rotations was 180 pounds mineral nitrogen per acre (instead of 240 pounds) and 140 pounds in form of stable manure (against 160 pounds as planned).

As far as the main crops are concerned, wheat started low with 7 bushels per acre, but rose soon to over 30 bushels; corn began with approximately 40 bushels per acre and was brought up to 50 to 60 bushels average; oats gave at first 20, later 60 bushels but the crop was more or less a failure in most years; potatoes were likewise unsatisfactory (with 80 to 150 bushels per acre); clover, too, gave good returns in two or three years only; whereas cowpeas, soybeans, and hairy vetch made invariably a good, or even excellent, growth.

The nitrogen data collected in table 1 give a clear picture of the success of the different treatments. In each case they are calculated in pounds per acre, so as to eliminate the disturbing influence of the varying number and size of plots to be compared.

Several facts stand out very clearly. The first nitrogen returns (in 1915) in cereals and potatoes, show that the average initial figures for all plots on field I, and on fields II and III, respectively, are as uniform as can be expected. The figures for fields II and III are twice as high as those for field I because of

TABLE 2  
*Nitrogen return in eight years*  
(Pounds per acre)

CROPS	NITROGEN					
	Above unmanured plots				Above legume plots	
	Mineral nitrogen*		Legumes	Legumes and stable manure†		
	lbs.	per cent	lbs.	lbs.	lbs.	per cent
Cereals and potatoes.....	71.5	39.7	67.2	121.0	53.8	38.4
Main crops.....	73.6	40.3	141.2	203.6	62.4	44.5
Catch crops.....	....	....	311.0	353.8	42.8	30.5
Total nitrogen return.....	73.6	40.3	452.2	557.4	105.2	75.0

\* 180 pounds N.

† 140 pounds N.

the differences in the quality of the soil. The data for 1923 and 1924 demonstrate that, despite the poorer quality of the soil on field I, the more suitable crop succession has raised the productivity to the same height as on fields II and III, and even higher on the plots that received organic manures. Already in 1916 and 1917 the effects of the different treatments were almost as well defined as they were again in 1923 and 1924, but the interruption caused a break which reduced to some extent the total figures for the eight years' rotations. Nevertheless, the superiority of the combined effect of growth of legumes and application of stable manure is very marked. The total figures for the respective plots on field I are almost identical with those for field II, where clover was grown on all plots for two years and stable manure, was applied uniformly. The plots on field I left without manure, as well as those receiving nothing but mineral

nitrogen or having only the benefit of legumes but no stable manure, are obviously inferior. No additional effect of the leguminous catch crops is noticeable on fields II and III, partly because of the poor growth of these crops and partly because of the predominating influence of the clover and of the stale manure. Because of the last named factor, the mineral nitrogen, too, has caused a very small crop increase.

Soybeans, cowpeas, and hairy vetch gave very regular and satisfactory nitrogen returns on field I (over 1,000 pounds nitrogen per acre for the full rotation), whereas the red clover on fields II and III failed almost completely in four out of eight years. Cowpeas and soybeans planted after wheat on fields II and III were likewise not reliable, (failures in 1917 and 1920); sweet clover proved equally unsatisfactory (in 1921 and 1922) and was therefore replaced by hairy vetch in 1923 and 1924.

The nitrogen recovered in the crops from the mineral and from the organic fertilizers applied on field I has been calculated in table 2.

As far as the main crops are concerned, a 40 per cent return is distinctly unsatisfactory for nitrate and ammonium sulfate, especially if practically the same return is obtained with stable manure. The 30 per cent return in the catch crops, however, raises the total for stable manure to 75 per cent, an unusually high figure, which shows the exceptional value of small applications of animal manure as well as the good effect of cover crops in preventing nitrogen losses by leaching. It might be concluded that the plots with mineral nitrogen would have given a better return if cover crops had been grown on them, too. To some extent this conclusion may be correct. But in view of the complete availability of the mineral nitrogen it is still more probable that the low returns are mainly due to the lack of organic matter in these plots and to the resulting insufficient carbon dioxide supply to the growing plants. That the percentage returns were markedly higher during the first part of the rotation—1916 to 1919: 20:60 per cent; 1922 to 1924: 20 per cent—points in the same direction.

The increase in nitrogen in cereals and in potatoes grown on the legume plots is about as high as that caused by the application of nitrate and ammonium sulfate. A percentage calculation is impossible because it is not known how much nitrogen has been added to the soil by the nitrogen-fixing bacteria of the legumes. But the 500 pounds (approximately) that have been harvested on these plots in eight years in excess of the 240 pounds on the unfertilized plots leaves no doubt that a vigorous bacterial action has taken place. The non-legume crops grown after legumes, have increased in all cases where the surface growth of the legumes has been removed. In only two years (1916 and 1917) did the plowing under of the vines give a little additional effect (12 and 15 per cent, respectively, above that secured from stubble and roots), which was almost within the limits of the fluctuations caused by inequalities of the soil.

If the total nitrogen in the stubble and roots of the legumes, is calculated at 250 pounds per 1,000 grounds surface growth, the percentage nitrogen return in cereals and potatoes grown on the legume plots would be approximately 27

per cent. This figure would fit very well those obtained in other experiments on the nitrogen availability in stubble and roots of legumes, but it is more probable that the increase in nitrogen return is not exclusively due to the decomposition of these crop residues.

Nitrogen analyses of the differently treated soil on field I made at the end of the rotation, gave the following average results: No manure, 0.055 per cent; legumes, 0.065 per cent; mineral nitrogen, 0.060 per cent; legumes and manure, 0.074 per cent.

In 1914 the average nitrogen content of this soil was 0.07 per cent. Accordingly, it seems as if the plots left without organic manure have lost a considerable amount of their nitrogen, whereas those receiving organic manures have remained practically constant, and that the large quantities of nitrogen harvested in the legumes have been drawn mainly from the air.

No marked change was noticeable, for fields II and III started with 0.1 per cent in 1914, and contained at the end of the rotation 0.091 per cent on the plots that received no other manure than stable manure and clover residues, and 0.099 per cent on those receiving in addition mineral nitrogen and leguminous catch crops, respectively.

#### *Greenhouse experiments*

In 1915 wheat was grown on some of the pots and peas on the remainder.<sup>2</sup> After harvesting, the pea vines, and in some cases their roots, were transferred to the wheat pots, so that comparative tests could be made. For further comparison, dried green manure was likewise used on the poor soil. Simultaneously on both soils, corn, kafir, and milo were grown, which were followed on the rich soil by another crop of corn. The nitrogen applied in the pea tops was 252 mgm. per pot on the poor soil and 410 mgm. on the rich soil. The nitrogen in the roots was not analyzed because the material available was just sufficient for the pot tests.

The data secured are shown in table 3.

The crops of corn, kafir, and milo after peas were not much higher than after wheat, although certain quantities of nitrogen had been removed in the wheat crops (30 mgm. on poor soil, 165 mgm. on rich soil). The increases due to the application of pea tops, either fresh or dry, are in the poor soil somewhat higher after peas than after wheat, whereas there is no marked difference on the rich soil. The pea pots on the rich soil, from which tops as well as roots were carefully removed, gave almost exactly the same returns as the wheat pots, where the crop residues were left. Pea soil plus pea residues produced a higher corn crop than pea soil without residues, but practically the same quantities of kafir and milo. The transferred pea tops and roots together produced about the same crops (dry weight) as the pea tops turned under after

<sup>2</sup> For the description of the pot tests, see the preceding article on "Nitrogen availability of green manures," in *Soil Sci.* 22: 253-290.

wheat, although the nitrogen content is markedly higher. Corn on poor soil returned 10 per cent nitrogen after wheat and 15 per cent after peas; kafir and

TABLE 3  
*Effect of pea growth, pea tops, and roots*

PREVIOUS CROP	TREATMENT	AVERAGE DRY WEIGHTS PER POT			N AVERAGE PER POT			
		Corn	Kafir	Milo	Corn	Kafir	Milo	
		gm.	gm.	gm.	mgm.	mgm.	mgm.	
Poor soil								
After wheat (30 mgm. N)	Wheat stubble only	7.0	5.0	5.6	44.6	47.1	43.1	
	Dried pea tops added	10.5	7.1	7.4	66.6	58.2	60.7	
	Fresh pea tops added	11.4	5.9	6.0	69.4	48.4	46.2	
	Maximum increase by pea tops	4.4	2.1	1.8	24.8	11.1	17.6	
After peas (252 mgm. N)	Pea tops and roots	18.6	9.0	9.2	98.6	71.5	64.4	
	Pea stubble and roots	10.2	5.5	7.0	59.2	45.3	49.0	
	Increase by tops	8.4	3.5	2.2	39.4	26.2	15.4	
Rich soil								
After wheat (165 mgm. N)	Wheat stubble only	1. Crop	14.4	2.7	3.1	145.8	43.6	45.0
		2. Crop	12.8	15.8	14.5	155.9	180.3	164.0
		Total	27.2	18.5	17.6	301.7	223.9	209.0
	Pea tops added	1. Crop	43.7	4.2	7.2	326.3	68.6	93.5
		2. Crop	14.3	24.3	23.0	139.6	255.3	220.8
		Total	58.0	28.5	30.2	465.9	323.9	314.3
		Increase by tops	30.8	10.0	12.6	164.2	100.0	95.3
	Pea tops and roots added	1. Crop	45.8	5.1	7.0	351.6	104.6	109.5
		2. Crop	14.3	28.1	23.8	150.1	295.5	267.6
		Total	60.1	33.2	30.8	501.7	400.1	377.1
		Above tops	2.1	4.7	0.6	35.8	76.2	61.8
After peas (410 mgm. N)	All residues re- moved	1. Crop	19.6	4.3	4.8	190.0	64.4	61.9
		2. Crop	11.3	15.7	14.7	117.5	170.4	167.0
		Total	30.9	20.0	19.5	307.5	234.8	228.9

TABLE 3—*Concluded*

PREVIOUS CROP	TREATMENT	AVERAGE DRY WEIGHTS PER POT			N AVERAGE PER POT		
		Corn	Kafir	Milo	Corn	Kafir	Milo
		gm.	gm.	gm.	mgm.	mgm.	mgm.

*Rich soil—continued*

After peas (410 mgm. N)—Con- tinued	Pea stubble and roots	1. Crop	25.8	4.6	6.8	263.6	61.9	85.0
		2. Crop	12.1	16.1	14.8	127.1	174.4	146.2
		Total	37.9	20.7	21.6	390.7	236.3	231.2
		Increase by roots	7.0	0.7	1.1	83.2	1.5	2.3
	Pea tops and roots	1. Crop	56.3	7.9	9.6	455.5	124.9	141.6
		2. Crop	13.7	24.1	22.6	140.9	204.5	189.7
		Total	70.0	32.0	32.2	596.4	329.4	331.3
		Increase by tops	32.1	11.3	10.6	205.7	93.1	100.1

milo gave still lower results. Rich soil furnished the following data for both crops:

	PER CENT N RECOVERED IN		
	Corn	Kafir	Milo
After wheat.....	40	24	25
After peas.....	50	23	24

The effect of growing legumes upon succeeding crops is undoubtedly not exclusively due to the manuring effect of their residues. Five other possible influences have to be considered, viz., increase of available potash and phosphate in the surface soil, slight increases in soil acidity, improved physical structure of the soil, suppression of weeds, and intensified bacterial action.

Crop rotation tests made in the greenhouse permitted the exclusion of all these possibilities except the last one. Regular alternating applications of potassium phosphate and of calcium carbonate supplied a surplus of available phosphate and potash and counteracted any possible increase in soil acidity.<sup>8</sup> No improvement in the physical structure of the soils took place in the pots. Every two years, the pots were emptied and the soil was thoroughly stirred. In the meanwhile, however, the texture, especially of the heavy clay soil, which was extremely poor in organic matter, became very bad.

<sup>8</sup> pH determinations made in 1925 gave for the poor soil 7.4 to 7.6, for the rich soil 6.6 to 6.8.

TABLE 4  
*Crop rotation experiments*  
Average dry weights per pot

DATE	CROP SUCCESSION	MISCELLANEOUS CROPS									
		None	Wheat	Rye	Oats	Corn	Field pea	Hairy vetch	Cow-pea	Soy-bean	
		gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	gms.	
<i>Poor soil</i>											
1920, September–November 1921, November–February 1921, February–May May–July October–December 1922, January–April April–June	1. Miscellaneous	....	4.2	3.4	3.5	19.0	20.4	7.8	10.7	11.4	
	2. Oats	5.6	2.6	2.4	2.3	2.7	7.1	5.5	4.6	2.9	
	3. Miscellaneous	....	2.1	1.7	2.3	4.9	17.7	18.5	22.7	15.2	
	4. Corn	7.5	6.8	6.7	6.5	7.0	20.5	17.6	14.0	8.8	
	5. Corn	9.2	10.2	10.3	9.9	10.7	11.5	10.5	9.4	10.0	
	6. Miscellaneous	....	3.8	3.3	4.6	12.9	16.7	15.2	13.9	20.9	
	7. Corn	7.9	8.2	8.0	7.7	8.1	9.3	13.1	10.4	10.3	
1920–22	(4) Corn (oats) crops	30.2	27.8	27.4	26.4	28.5	48.4	46.7	38.4	32.0	
	(3) Miscellaneous crops	....	10.1	8.4	10.4	36.8	54.8	41.5	47.3	48.5	
	Total	30.2	37.9	35.8	36.8	65.3	103.2	88.2	85.7	80.5	
1923, November–January 1924, February–May June–July September–October November–February 1925, March–April May–June	1. Corn	13.3	12.8	12.8	13.2	13.1	13.6	13.5	13.4	13.1	
	2. Miscellaneous	....	3.6	4.1	7.3	8.4	49.8	38.6	28.6	15.6	
	3. Corn	8.4	6.4	6.4	6.1	7.0	19.4	24.5	14.2	8.2	
	4. Corn	8.0	7.8	7.7	8.9	8.2	10.1	9.9	9.4	7.7	
	5. Miscellaneous	....	1.6	1.8	2.0	1.9	5.9	4.9	6.3	4.2	
	6. Corn	5.9	3.7	3.7	3.5	4.7	12.6	8.3	8.8	4.9	
	7. Corn	3.4	3.7	3.5	3.9	3.8	4.3	4.3	4.0	3.4	
1923–25	(5) Corn crops	39.0	34.4	34.1	35.6	36.8	60.0	60.5	49.8	37.3	
	(2) Miscellaneous crops	....	5.2	5.9	9.3	10.3	55.7	43.5	34.9	19.8	
	Total	39.0	39.6	40.0	44.9	47.1	115.7	114.0	84.7	57.1	

*Rich soil*

1923, November-January	1. Corn	14.4	13.6	13.9	13.5	13.7	13.6	13.4	13.4	13.4
1924, February-May	2. Miscellaneous	....	8.0	7.0	11.5	17.3	37.4	28.6	21.6	7.2
June-July	3. Corn	17.4	10.2	10.7	9.6	10.4	27.2	28.8	21.9	12.9
September-October	4. Corn	6.9	6.9	6.6	6.9	6.2	8.0	7.6	7.9	5.7
November-February	5. Miscellaneous	....	2.0	2.0	2.4	1.4	6.7	4.5	5.5	4.0
1925, March-April	6. Corn	8.9	5.5	5.7	5.7	8.5	15.9	11.8	11.0	6.0
May-June	7. Corn	5.0	5.1	5.2	5.2	4.8	5.9	5.9	5.2	5.1
1923-25	(5) Corn crops	52.6	41.3	42.1	40.9	43.6	70.6	67.5	59.4	43.1
	(2) Miscellaneous crops	....	10.0	9.0	13.9	18.7	44.1	33.1	27.1	11.2
	Total	52.6	51.3	51.1	54.8	62.3	114.7	100.6	86.5	54.3



TABLE 5

*Crop rotation experiments*

Average nitrogen returns per pot

DATE		CROP SUCCESSION		MISCELLANEOUS CROPS																	
		None		Wheat		Rye		Oats		Corn		Field pea		Hairy vetch		Cow-pea		Soy-bean			
		mgm.		mgm.		mgm.		mgm.		mgm.		mgm.		mgm.		mgm.		mgm.			
<i>Poor soil</i>																					
1920, September–November November–February 1921, February–May May–July October–December 1922, January–April April–June	1. Miscellaneous	.....	55.8	51.2	53.8	173.1	694.5	307.4	380.2	243.9											
	2. Oats	60.0	42.6	41.0	36.8	41.9	103.7	69.9	63.9	46.7											
	3. Miscellaneous	.....	29.4	27.5	30.8	37.2	584.1	573.5	703.7	408.3											
	4. Corn	52.5	54.4	54.3	54.6	53.9	149.7	117.9	100.8	67.8											
	5. Corn	106.7	109.1	106.1	109.9	119.8	130.0	119.7	110.0	118.0											
	6. Miscellaneous	.....	57.4	64.4	68.1	120.0	287.2	621.7	415.6	844.6											
	7. Corn	53.7	67.2	50.4	50.8	55.9	64.2	87.8	70.7	74.2											
1920–22	(4) Corn (oats) crops	272.7	273.3	251.8	252.1	271.5	447.6	395.3	345.4	306.7											
	(3) Miscellaneous crops	.....	142.6	142.1	152.7	330.3	1,565.8	1,108.6	1,499.5	1,696.8											
	Total	272.7	415.9	393.9	404.8	601.8	2,013.4	1,497.9	1,844.9	2,003.5											
1923, November–January 1924, February–May June–July September–October November–February 1925, March–April May–June	1. Corn	151.6	145.9	145.9	150.5	149.3	152.8	153.9	155.0	149.3											
	2. Miscellaneous	.....	42.5	46.7	84.7	84.0	1,394.4	1,494.5	1,086.8	346.3											
	3. Corn	52.1	44.8	51.2	50.0	51.1	110.6	181.3	95.1	57.4											
	4. Corn	71.2	68.8	67.8	77.4	79.5	90.9	89.1	84.6	71.1											
	5. Miscellaneous	.....	30.2	38.2	35.6	41.8	260.8	220.5	247.0	110.0											
	6. Corn	48.4	34.8	35.2	35.7	42.3	100.8	74.7	70.4	42.1											
	7. Corn	27.2	31.8	27.3	31.2	31.5	36.1	35.3	32.4	29.9											
1923–25	(5) Corn crops	350.5	326.1	327.4	344.8	353.7	491.2	534.3	437.5	349.8											
	(2) Miscellaneous crops	.....	72.7	84.9	120.3	125.8	1,655.2	1,715.0	1,333.8	456.3											
	Total	350.5	398.8	412.3	465.1	479.5	2,146.4	2,249.3	1,771.3	806.1											

*Rich soil*

1923, November-January 1924, February-May June-July September-October November-February 1925, March-April May-June	1. Corn 2. Miscellaneous 3. Corn 4. Corn 5. Miscellaneous 6. Corn 7. Corn	195.8	185.0	189.0	183.6	186.3	185.0	182.2	182.2	182.2
		.....	78.4	81.2	89.7	128.0	921.0	966.7	781.9	103.7
1923-25		120.0	78.5	76.0	72.0	77.0	204.0	239.0	164.3	95.5
		80.4	74.5	74.6	81.4	73.2	92.0	99.6	87.7	66.1
		.....	42.8	43.6	48.0	33.0	257.3	189.0	215.6	71.2
		78.3	53.4	55.3	57.0	84.2	133.6	103.8	99.0	56.4
		40.0	40.8	41.1	42.6	39.8	46.6	49.6	44.7	41.3
1923-25	(5) Corn crops	514.5	432.2	436.0	436.6	460.5	661.2	674.2	577.9	441.5
	(2) Miscellaneous crops	.....	121.2	124.8	137.7	161.0	1,178.3	1,155.7	997.5	174.9
	Total	514.5	553.4	560.8	574.3	621.5	1,839.5	1,829.9	1,575.4	616.4

To eliminate any trace of the previous differential treatments, all pots were emptied, their contents thoroughly mixed, and one or two test crops grown. The first set of rotation experiments was made on only the poor soil from September, 1920 to June, 1922. A second set was run simultaneously on both soils from November, 1923 to June, 1925.

In every case four or five evenly distributed rows of eight pots were planted in regular alternation to corn (oats, in one case) and to miscellaneous crops. The latter comprised four cereals (wheat, rye, oats, and corn) and four legumes (Canada field pea, hairy vetch, cowpea, and soybean). In addition there were five rows not planted when the miscellaneous crops were grown, but corn was raised on them as on all other rows.

Table 4 shows the average dry weights of the different crops, and table 5 the nitrogen contained therein.

These rotations were started with corn. The very uniform data obtained show the accuracy of these average figures, which are based on 32 single determinations (in the no-crop series, on 40). Seasonal influences, however, cause wide fluctuations, but if crops grown at the same season in different years are compared, remarkable uniformity is noticeable. This may be seen from the data for corn grown on the poor soil in May to July, 1921 and in June and July, 1924.

A comparison of the total figures for dry weights and for nitrogen harvested in the seven crops shows in every case that all cereal rows, with the exception of the dry weight for the corn row in the first test, were far below those recorded for the legume rows. The latter were generally two to three times higher in dry weight, and three to five times higher in nitrogen, with the exception of the soybean rows in the last two tests. If no crops were grown between the corn crops, the result was always below that of the rows planted continuously to corn. The most interesting fact is that the total crops in cereals were not lower—in the last two tests they were even distinctly higher—than in the permanent cereal rows. Four or five corn crops grown under the influence of legumes were equal or superior to seven corn and small grain crops grown without legumes.

The two crops of legumes on the poor soil in 1924 were about equal to the three crops raised on the same soil two years earlier, and they were markedly higher in dry weight and nitrogen. This originally rich soil which, 10 years earlier, produced much heavier crops than the poor soil, has now, after 23 crops, almost reached the same low level of productivity. The better growth of legumes on the poor soil will probably soon establish complete uniformity for both soils under this system of crop succession. Already the characteristic differences in the behavior of the four legumes tested are very marked and uniform on both soils. Field peas exert the most beneficial effect, followed by vetch and cowpeas, whereas soybeans are regularly more or less inferior. It must be emphasized in this connection, that the first three plants are usually still green when cut, whereas the soybeans are ripe. In the first test the field peas also (January to April, 1922) produced ripe seed, and there was, indeed,

TABLE 6  
*Influence of time upon after-effect to corn*

RICH SOIL*										RICH SOIL†										POOR SOIL‡									
INTER- VAL	Dry weights					Nitrogen					INTER- VAL	Dry weights					Nitrogen												
	After no crop		After pea		+ -		After no crop		After pea			+ -		After no crop		After pea		+ -											
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.		gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.										
0	11.8	11.8	0	0	113.0	118.0	5.0	11.2	12.4	1.2	80.6	86.8	6.2	0	9.7	11.8	2.1	69.9	82.6	12.7									
1	16.9	18.0	1.1	1.1	136.0	144.9	8.9	13.1	17.4	4.3	86.5	114.8	28.3	2	10.4	12.6	2.2	60.3	85.7	25.4									
2	18.1	20.1	2.0	2.0	162.4	172.8	10.4	14.9	17.9	3.0	93.9	114.6	20.7	4	10.5	12.5	2.0	67.2	80.0	12.8									
3	22.1	24.0	1.9	1.9	172.4	188.2	15.8	19.2	23.6	4.4	119.0	146.3	27.3	6	11.9	14.4	2.5	83.3	86.4	3.1									
4	23.1	25.9	2.8	2.8	174.6	190.9	16.3	21.6	34.8	13.2	138.2	208.8	70.6	8	15.0	19.6	4.6	78.0	105.8	27.8									
5	23.5	26.3	3.8	3.8	205.2	224.6	19.4	38.1	50.0	11.9	221.0	310.0	89.0	10	21.8	28.1	6.3	104.6	157.4	52.8									
6	27.0	31.4	4.4	4.4	216.0	242.3	26.3	26.6	33.3	6.7	170.2	213.1	42.9	12	14.8	18.3	3.5	94.7	120.8	26.1									
7	30.5	34.2	3.7	3.7	225.7	257.9	32.2	26.4	30.4	4.0	184.8	237.1	52.3	14	16.4	19.8	3.2	118.1	142.6	24.5									
8	33.0	38.5	5.5	5.5	236.0	274.6	38.6	21.8	26.5	4.7	222.4	238.5	16.1	16	14.0	17.7	3.7	123.2	141.6	18.4									
9	37.6	45.5	7.9	7.9	240.2	297.0	56.8	....	....	....	....	....	....	..	....	....	....	....	....	....									
Average.....			3.3		....	....	23.0	....	....	6.0	....	....	39.3	..	....	....	3.3	....	....	22.6									

\* Corn planted April 1 to June 9, harvested June 7 to August 10, 1922.

† Corn planted April 26 to August 30, harvested June 28 to November 1, 1923.

very little increase in the following corn crop. It is true that the total nitrogen in this pea crop was unusually low, but this does not fully explain the situation. The total nitrogen harvested in the various leguminous crops frequently has no relation to the effect upon the next corn crop. Cowpeas, for instance, gave higher nitrogen returns than vetch during September to November, 1920, February to May, 1921, and November to February, 1924, on both soils, but the lower corn crops always came after cowpeas. During January to April, 1922, the nitrogen in soybeans was much higher than in vetch, but again the soybeans showed the lower after-effect.

When two corn crops were grown after legumes the after-effect was almost negligible in the second crop, but there was likewise a low effect upon the first corn crop noticeable if this was planted very soon after the legumes were harvested. This was the case with the last corn crop grown in the first test (April to June, 1922). As a rule the pots were replanted about four weeks after the previous crops had been cut, but in this case the corn was sown within a week. Some special tests in this direction seemed necessary.

After thorough mixing and preliminary testing of the soil, alternating rows of eight pots were planted throughout the house to corn and to peas, respectively, and the crops were removed. Immediately afterward, four rows evenly distributed through the house were replanted to corn, two after corn and two after peas. This was repeated at weekly intervals until after nine weeks all 36 rows were replanted. The harvesting was done in the same succession after two months. (See table 6.) There was no difference in dry weight and very little in nitrogen, immediately after the preceding crops were removed, but there was a gradual increase in the differences in dry weight and still more in nitrogen up to the end of the test. Accordingly, this experiment was repeated the next year on both soils, but this time two-week intervals were chosen in order to secure more complete data. (See table 6.) Again the differences were very small in the beginning, they reach their maximum after eight to ten weeks, and then they decline; but even after sixteen weeks they were considerably larger than at the beginning, and although they rise and fall generally with the crop weights at the different times of the year, there are still certain exceptions to this rule. This is especially noticeable with the total nitrogen returns at the end of the tests. After a temporary decline a second maximum is reached in the fall, which is evidently due to the increase in soil nitrification. The differences between the nitrogen returns also decline rapidly, and if the tests could have been continued, probably uniform results would soon have been obtained.

That the larger crops harvested after legumes are not due to an increased consumption of nitrogen originally present in the soil was indicated by the chemical analyses made at the end of the eight-year crop rotations in the field. The plots on which legumes were grown always showed the highest nitrogen percentage. The analyses of the soils in the pots furnish confirmative and more decisive data. The original nitrogen content in 1915 was 0.103 per cent

in the poor soil and 0.143 per cent in the rich soil. At the end of the second rotation in 1925, the following results for the differently treated rows were obtained (per cent N, average of four rows):

	NO CROP	WHEAT	RYE	OATS	CORN	PEA	VEITCH	COWPEA	SOYBEAN
Poor soil.....	0.059	0.062	0.061	0.058	0.062	0.062	0.063	0.062	0.060
Average ....		Cereals: 0.061				Legumes: 0.062			
Rich soil.....	0.107	0.108	0.108	0.106	0.109	0.108	0.105	0.105	0.105
Average ....		Cereals: 0.108				Legumes: 0.106			

The small differences in the nitrogen content of cereal and legume rows are within the fluctuations always to be found in such soil tests. (A deviation of 0.001 per cent is equivalent to 90 mgm. nitrogen per pot—20 pounds soil.) Since both soils were uniformly mixed before the last rotation experiment was started, in order to eliminate any possible influence of the preceding experiments, such uniform results were to be expected. Marked differences, however, will become noticeable after these crop rotations will have been repeated several times. At present two facts stand out very clearly: (a) The original nitrogen content has been lowered to about three-fifths in the poor soil, and to three-fourths in the rich soil; (b) the soil under legumes is not lower in nitrogen, despite the larger amounts removed in the crops.

A complete nitrogen balance (per pot) for both soils presents the following picture, if it is based upon the data recorded for the crops grown on the (unmanured) check rows in the green manuring experiments, and on those raised in the permanent corn rows in the rotation experiments or in special tests as discussed before:

NITROGEN REMOVED				
	From poor soil		From rich soil	
		mgm.		mgm.
Green manuring experiments.....	11 crops	810	8 crops	1,640
1. Rotation and special tests.....	11 crops	1,010	8 crops	1,250
2. Rotation experiments.....	7 crops	480	7 crops	620
Total.....	29 crops	2,300	23 crops	3,510
Original nitrogen in 20 pounds = 9000 grams soil.....	(0.103%)	9,270	(0.143%)	12,870
Added in water*.....		250		500
Green manure residues†.....	(50%)	150	(20%)	90
Total nitrogen available.....		9,670		13,460

	NITROGEN REMOVED			
	From poor soil		From rich soil	
		mgm.		mgm.
Soil nitrogen at end of experiment.....	(0.061%)	5,490	(0.107)	9,630
Reduction in soil nitrogen.....		4,180		3,830
Removed in crops.....		2,300		3,510
Difference.....		1,880		320
Equivalent to a loss in original nitrogen of....		20%		2.4%

\* Since the water sprayed on the pots has not been measured, the nitrogen added in it has been calculated from its analysis (0.4 p.p.m. total nitrogen) and from the high estimate that 500 parts water were used for the production of 1 part dry matter. The 29 crops on poor soil contained 1010 gm., the 23 crops on rich soil, 2116 gm. dry matter.

† The green manures applied averaged 300 mgm. N per pot. Since only 50 per cent of it was taken up by the manured crops on poor soils and 80 per cent on rich soil, and later in mixing the soil the residues were evenly distributed in all pots, the remaining nitrogen had to be added to the original soil nitrogen.

The small difference found for the rich soil is insignificant (an analytical deviation of 0.004 per cent nitrogen is equivalent to 360 mgm. nitrogen per pot), but there is no doubt that large losses have occurred in the poor soil. The bad physical character of the heavy clay favors denitrification, which probably has also been mainly responsible for the imperfect action of the green manure nitrogen in this soil.

A comparison of the nitrogen harvested on the poor soil in the permanent corn experiments and in those wherein corn alternated with peas, presents the following picture:

	NITROGEN REMOVED	
	By corn after corn	By corn and peas
	mgm.	mgm.
1. Rotation and special tests.....	1,010	2,420
2. Rotation.....	480	2,150
Total.....	1,490	4,570
More nitrogen in the corn-pea rotations.....	(18 crops)	3,080

Compared with the loss of 1880 mgm. nitrogen in the permanent corn experiment, a gain of 1200 mgm. nitrogen is to be recorded for the corn-pea series. Accordingly, an average gain in nitrogen from the air was obtained by the growth of one crop of peas equivalent to 600 mgm. per pot or approximately 200 pounds per acre (calculated as 3 million pounds of soil). The excellent

growth of the peas under greenhouse conditions is the cause of these high figures, which indicate the possibilities offered by a frequent return of legumes in crop rotations, although these possibilities can not be fully realized in the field.

### *Laboratory experiments*

During the last two years laboratory experiments were made regularly and in a fairly comprehensive manner in conjunction with the last crop rotation experiments on both soils, and to some extent with the field experiments.

Thoroughly mixed soil samples taken at frequent intervals from differently treated pots or plots were used for the following determinations:

- (a) Total counts of colonies grown on soil extract agar.
- (b) Counts of colonies of the *Radiobacter* group on nitrate glycerin agar.
- (c) Counts of colonies of *Actinomycetes* on the same agar.
- (d) Counts of mold colonies on acid nitrate dextrose agar.
- (e) Nitrates produced in 20 days in 50 cc. 0.1 per cent ammonium phosphate solution inoculated with 5 gm. soil.
- (f) Nitrogen assimilated from the air in 10 days in 100 cc. 1 per cent mannite solution inoculated with 10 gm. soil.
- (g) Amount of nitrates present in the soil samples.

Details about the methods used have been published elsewhere (23a, 32). The acid nitrate dextrose agar was prepared after the following formula: 1000 cc. soil extract, 1 gm.  $\text{KH}_2\text{PO}_4$ , 1 gm.  $\text{KNO}_3$ , 10 gm. dextrose, 30 gm. agar, adjusted to pH 4.0 to 4.5 by mixture of hydrochloric and sulfuric acid.

The nitrates present in the soil were determined by the phenol-disulfonic-acid method. The extract of 100 gm. soil was clarified by adding 50 cc. of a suspension of  $\text{Al}(\text{OH})_3$  before filtering.

The results presented in tables 7 to 9 are based upon the data obtained with four composite samples of the differently treated soils (two from poor, two from rich soil), each of them prepared from the samples taken in about 4 inches depth from the eight pots of every row. Four plates were poured in every case; the figures given for each soil are, therefore, the average of eight single determinations. The great accuracy of the averages based upon these figures is clearly demonstrated by the uniform figures obtained for the total counts of bacteria, of actinomycetes, and of fungi, at the beginning and at the end of the experiment, respectively.

On account of the conspicuous fluctuations in the total bacterial counts the results recorded in table 7 are given separately for every sampling, whereas in tables 8 and 9 merely average results have been inserted. As not all differently planted rows could be sampled simultaneously, the most important crops have been selected for these examinations.

The total bacterial counts under the different crops as given in table 7 and shown graphically in figure 1, leave no doubt about the very pronounced influence exerted by most of the legumes upon the soil microflora. The curves



TABLE 7  
*Number of colonies on soil extract agar*  
 Millions per gram soil  
 (P = poor soil, R = rich soil, A = average count)

SAMPLING	NO CROPS			CORN			WHEAT			SOYBEAN (1924) VETCH (1925)			COWPEA			FIELD PEA (1925)		
	P		R		A		P		R		A		P		R		P	
	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A
February 4, 1924.....	68	38	53	64	38	51	61	43	52	68	40	54	70	37	51	...	...	...
February 25, 1924.....	47	60	54	62	43	53	53	64	58	55	47	51	51	73	62	...	...	...
Before planting.....	58	49	54	63	41	52	57	54	55	62	44	53	60	55	57	...	...	...
March 17, 1924.....	39	58	48	70	78	74	59	62	59	53	55	54	52	67	60	...	...	...
April 1, 1924.....	58	39	49	86	67	76	82	50	66	63	44	54	68	53	60	...	...	...
April 28, 1924.....	44	36	40	66	59	62	57	49	53	45	45	45	59	55	57	...	...	...
May 14, 1924.....	56	48	52	68	53	60	67	43	55	52	47	50	60	55	57	...	...	...
Under various crops.....	49	45	47	72	64	68	66	51	58	53	48	51	60	58	59	...	...	...
June 16, 1924.....	57	52	55	54	64	59	68	70	69	58	56	57	99	188	144	...	...	...
July 22, 1924.....	146	30	88	65	51	58	62	56	59	81	60	71	100	110	105	...	...	...
Under corn 1.....	101	41	71	60	58	59	65	63	64	70	58	64	100	147	124	...	...	...
October 20, corn 2.....	38	25	32	52	37	45	61	38	50	74	40	57	74	47	60	...	...	...
Averages, 1924.....	61	45	53	65	55	60	63	53	58	61	49	55	70	76	73	...	...	...

December 8, 1924.....	57	18	38	88	51	70	88	51	70	127	66	97	117	76	97	133	119	126
December 20, 1924.....	54	30	42	79	63	71	86	48	67	121	66	94	65	59	62	152	122	137
January 12, 1925.....	49	37	43	45	32	39	54	50	52	81	60	71	81	46	64	95	78	87
January 26, 1925.....	47	27	37	55	46	51	56	36	46	83	48	66	63	47	55	165	116	145
Under various crops.....	52	28	40	67	48	58	71	46	59	103	60	82	81	57	69	136	109	123
March 23, 1925.....	40	22	31	64	42	53	57	64	61	76	60	68	86	60	73	97	93	95
April 20, 1925.....	42	26	34	56	42	51	56	45	51	90	51	71	96	61	79	73	57	65
Under corn 1.....	41	24	33	60	42	52	57	55	56	83	56	70	91	61	76	85	75	80
June 6, corn 2.....	60	52	56	76	55	66	76	53	65	108	57	83	99	83	91	115	61	88
August 3, bare.....	38	28	33	34	25	30	32	31	32	40	30	35	38	27	33	49	35	42
Averages, 1925.....	48	30	39	62	45	54	63	45	54	91	55	73	80	58	69	110	85	98

TABLE 8  
*Number of colonies of the radiobacter group and of actinomycetes, grown on glycerin nitrate agar, and colonies of fungi, grown on acid dextrose nitrate agar*  
 Actinomycetes, millions per gram soil  
 Radiobacter group and fungi, 10,000 per gram soil

SAMPLING	NO CROP			CORN			WHEAT			SOYBEAN (1924) VETCH (1925)			COWPEA			FIELD PEA (1925)			
	P		R	A	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A
<i>Radiobacter group</i>																			
Before planting.....	50	50	50	120	70	95	100	44	72	90	60	75	100	65	83	...	...	...	...
Under various crops.....	20	10	15	80	40	60	70	50	60	50	50	50	111	111	111	...	...	...	...
Corn 1, 1924.....	30	10	20	50	40	45	50	50	50	70	40	55	240	260	250	...	...	...	...
Corn 2, 1924.....	30	0	15	40	40	40	60	20	40	70	30	50	80	60	70	...	...	...	...
Under various crops.....	12	1	7	61	65	63	59	35	47	95	42	69	114	77	95	158	253	206	...
Corn 1, 1925.....	38	3	21	69	54	61	83	103	93	124	76	100	171	84	122	173	133	153	...
Corn 2, 1925.....	141	18	80	56	43	50	86	45	66	155	58	107	243	54	149	203	25	114	...
Bare, dry.....	8	5	7	30	0	15	15	5	10	30	13	22	28	8	18	30	8	19	...
Averages.....	35	15	25	70	50	60	66	47	57	66	46	56*	132	100	116	151	161	156	...
										102	50	76							
<i>Actinomycetes</i>																			
Before planting.....	7.0	6.8	6.9	7.5	6.3	6.9	7.6	6.0	6.8	8.3	6.9	7.6	7.9	7.1	7.5	...	...	...	...
Under various crops.....	8.8	5.2	7.0	10.9	6.5	8.7	10.6	7.0	8.8	9.2	6.6	7.9	9.4	6.8	8.1	...	...	...	...
Corn 1, 1924.....	8.4	5.6	7.0	8.9	5.7	7.3	9.1	5.3	7.2	10.5	5.7	8.1	12.2	5.5	8.8	...	...	...	...
Corn 2, 1924.....	10.9	5.2	8.0	11.1	4.9	8.0	11.5	5.5	8.5	12.5	5.6	9.0	14.3	6.9	10.6	...	...	...	...
Under various crops.....	9.0	4.2	6.6	11.0	5.2	8.0	11.3	4.4	7.8	13.5	5.1	9.2	12.3	4.9	8.6	15.1	5.8	10.5	...
Corn 1, 1925.....	8.7	3.8	6.6	10.2	5.3	7.7	10.7	3.6	7.3	12.5	4.9	8.7	14.1	4.4	9.3	13.4	4.9	9.1	...
Corn 2, 1925.....	12.3	3.9	8.1	11.3	4.1	7.7	12.3	4.7	8.5	12.7	4.8	8.8	11.3	4.1	7.7	13.1	6.0	9.6	...
Bare, dry.....	9.4	5.4	7.4	9.7	4.9	7.3	8.3	5.6	7.6	10.2	6.8	8.5	10.9	6.4	8.7	10.8	6.7	8.8	...
Averages.....	9.0	5.0	7.0	11.2	5.6	8.4	10.2	5.4	7.8	9.5	6.3	7.9*	11.2	6.4	8.8	13.9	5.7	9.8	...
										14.0	5.2	9.6							

F<sub>400</sub>

Before planting.....	14	19	17	24	19	22	22	17	19	24	17	20	27	17	22	..	..
Under various crops.....	11	21	16	18	18	18	17	17	17	20	20	20	20	20	20	..	..
Corn 1, 1924.....	14	21	17	21	25	23	19	21	20	21	20	21	26	19	23	..	..
Corn 2, 1924.....	10	13	12	25	22	24	33	28	31	32	38	35	31	34	33	..	..
Under various crops.....	9	10	10	24	22	23	30	22	26	31	33	32	30	28	29	34	32
Corn 1, 1925.....	8	10	9	27	29	28	40	30	35	43	45	44	48	27	38	42	41
Corn 2, 1925.....	38	30	34	28	20	24	35	22	29	45	30	38	33	56	45	35	36
Bare, dry.....	9	28	19	8	23	16	10	21	16	7	27	17	10	33	22	12	20
Averages.....	12	17	15	22	21	22	25	21	23	23	21	22*	29	27	28	33	33
										32	35	34					

\* 1924 soybeans, 1925 vetch.

TABLE 9

*Asotobacter* tests (mgm. N assimilated in 10 days in 1 per cent mannite solution), nitrification tests (mgm. nitrified in 20 days in 0.1 per cent ammonium phosphate solution), and nitrate determinations in soils (nitrate nitrogen, parts per million)

SAMPLING	NO CROP			CORN			WHEAT			SOYBEAN (1924) VECTEE (1925)			COWPEA			FIELD PEA (1925)			
	P		R	A	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A
	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A	P	R	A	P
Milligrams N assimilated																			
Before planting.....	8.1	9.3	8.7	9.0	9.1	9.1	8.6	9.1	8.9	8.7	8.9	8.8	9.0	9.1	9.1	....	....	....	....
Under various crops.....	8.0	9.0	8.5	8.7	9.6	9.2	8.0	9.4	8.7	8.0	9.9	9.0	8.0	9.5	8.8	....	....	....	....
Corn 1, 1924.....	9.0	9.0	9.0	8.7	8.8	8.8	8.9	9.1	9.0	9.2	8.4	8.8	8.9	7.4	8.2	....	....	....	....
Corn 2, 1924.....	9.2	11.7	10.5	9.8	10.7	10.2	9.1	8.4	8.8	9.6	9.4	9.5	9.4	8.5	9.0	....	....	....	....
Under various crops.....	8.2	10.5	9.4	8.8	11.0	9.9	9.4	11.3	10.3	9.6	10.4	10.0	9.0	9.6	9.3	9.1	9.9	9.5	9.5
Corn 1, 1925.....	9.5	9.0	9.3	9.2	10.6	10.0	10.4	10.3	10.4	11.0	10.8	10.9	9.6	8.8	9.2	9.6	9.1	9.4	9.4
Corn 2, 1925.....	10.6	9.6	10.1	11.4	10.4	10.9	11.6	11.5	11.6	12.4	8.2	10.3	12.8	9.2	11.0	11.4	11.6	11.5	11.5
Bare, dry.....	8.9	9.2	9.1	8.9	9.1	9.0	9.5	9.6	9.6	9.8	9.4	9.6	10.6	9.4	10.0	9.5	8.8	9.2	9.2
Averages.....	8.6	9.5	9.1	9.0	9.8	9.4	9.1	10.0	9.6	8.6	9.3	9.0	9.2	9.2	9.2	9.6	9.8	9.8	9.7
Milligrams N nitrified																			
Before planting.....	6.9	5.4	6.2	7.0	6.0	6.5	6.6	5.8	6.2	6.7	5.7	6.2	5.7	5.5	5.6	....	....	....	....
Under various crops.....	7.0	5.6	6.3	6.6	5.7	6.2	6.5	5.5	6.0	6.8	5.5	6.2	7.0	5.6	6.3	....	....	....	....
Corn 1, 1924.....	6.5	5.7	6.1	6.4	4.5	5.4	6.5	4.3	5.4	6.7	5.1	5.9	7.2	5.5	6.4	....	....	....	....
Corn 2, 1924.....	5.1	5.4	5.2	2.9	4.7	3.8	4.8	4.8	4.8	4.4	4.3	4.4	5.7	5.1	5.4	....	....	....	....
Under various crops.....	6.7	5.6	6.1	6.5	3.5	5.0	6.3	3.7	5.0	7.1	4.2	5.7	7.5	4.6	6.0	7.8	5.4	6.6	6.6
Corn 1, 1925.....	5.1	4.7	4.9	6.2	3.2	4.7	6.2	2.9	4.6	6.6	4.0	5.3	6.9	4.4	5.7	7.1	4.6	5.9	5.9
Corn 2, 1925.....	6.0	5.7	5.9	5.8	6.2	6.0	5.7	6.1	5.9	6.2	6.9	6.6	6.0	6.5	6.3	6.1	6.7	6.4	6.4
Bare, dry.....	6.6	5.4	6.0	5.8	5.2	5.5	5.7	5.1	5.4	6.3	5.4	5.9	6.5	4.9	5.7	7.0	5.5	6.3	6.3
Averages.....	6.4	5.4	5.9	6.2	4.6	5.4	6.2	4.6	5.4	6.5	5.2	5.9	6.8	5.1	6.0	7.0	5.5	6.3	6.3

# EFFECT OF GROWING LEGUMES UPON SUCCEEDING CROPS

*Nitrate N in soils*

Under various crops.....	11.4	11.6	11.5	3.0	3.8	3.4	2.9	2.4	2.7	4.0	3.6	3.8	1.6	2.6	2.1	5.6	7.9	6.8
Corn 1, 1925.....	13.3	22.3	17.8*	1.3	2.7	2.1	1.1	1.4	1.3	2.5	3.3	2.9	1.3	2.8	2.1	3.4	3.9	3.7
Corn 2, 1925.....	1.1	1.2	1.2	2.1	1.3	1.7	2.0	1.6	1.8	3.8	1.5	2.7	3.2	2.0	2.6	3.0	1.5	2.2
Bare, dry.....	5.2	9.4	7.3	7.8	12.8	10.3	7.0	12.0	9.5	7.4	14.5	11.0	7.1	11.7	9.4	7.1	13.5	10.3
Averages.....	12.3	17.2	14.8*	3.1	4.5	3.8	2.8	3.6	3.2	4.0	5.0	4.5	2.7	4.1	3.4	4.7	6.4	5.6
	3.1	5.3	4.2															

\* The first two nitrate determinations were made with soil which was kept bare permanently. The last two determinations, however, were made with soil that was planted to corn after it had remained bare while miscellaneous crops were grown on the other plots.

for field pea, vetch, and cowpea stand out conspicuously, whereas the results obtained under and after soybeans are not very different from those found with cereals. In their effect upon the succeeding crops the legumes tested ranked as follows: peas, vetch, cowpea, soybean. The averages of the bacterial counts are for pea 98, vetch 73, cowpea 69, and soybean 55 millions, a very close coincidence. However, the wide fluctuations recorded for the various dates demonstrate clearly that decisive results can not be expected unless such tests are made upon a broad basis and for long periods. In addition, a comparison of the numbers of bacteria, as well as of actinomycetes, found in both soils leaves no doubt that there exists no direct correlation between the number of soil bacteria and soil productivity. As in the present case, an increase in the number of soil organisms may accompany, or in part even cause an increase

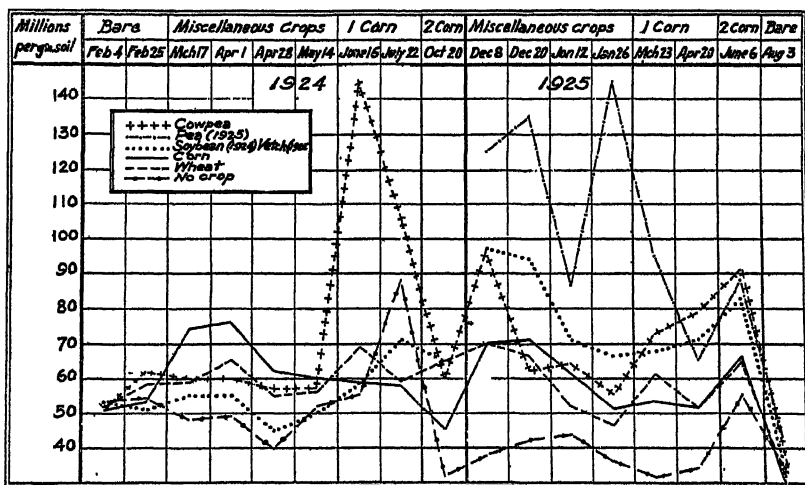


FIG. 1. TOTAL BACTERIAL COUNTS UNDER AND AFTER DIFFERENT CROPS GROWN IN POT EXPERIMENTS

in, soil productivity, but the fertility of different soils is dependent on so many other factors that no uniform correlations can be expected (23, p. 513).

Actinomycetes, as well as molds, were slightly more numerous under and after legumes than under cereals. Very wide differences occurred within the so-called Radiobacter group. (Table 8.) These organisms play an important rôle in the soil, because of their ability to assimilate elementary nitrogen as well as nitrate nitrogen. They were much more numerous under and after field peas and cowpeas than under cereals, whereas they were almost absent in the no-crop series, despite the high nitrate content of these soils. (Table 9.) A special experiment was made in order to ascertain whether an increase in Radiobacter growth could be induced by adding straw to these soils rich in nitrate. With the poor soil, positive results were obtained, whereas in the rich soil a marked stimulation in mold growth took place. Since many members

of the last named group are also able to assimilate nitrate nitrogen very rapidly under suitable conditions, this result is not surprising. The different behavior of the two soils is explained by the fact that the poor soil is alkaline (pH 7.4 to 7.6), whereas the rich soil is slightly acid (pH 6.6 to 6.8).

At present it is impossible to decide definitely whether the activity of members of the Radiobacter group under and after legumes is greater in nitrate assimilation or in the fixation of elementary nitrogen. The last named activity, however, probably predominates. Table 9 shows that the soils contained more nitrate nitrogen under and after legumes (especially pea and

TABLE 10  
*Average results of bacteriological and biochemical tests of field soils*

		FIELD I			ORIGINAL SOD BETWEEN FIELDS I/II	FIELD II	
		Corn	Cowpea	Soybean		Wheat	Vetch followed by potatoes
Bacterial counts, millions per gram soil.....	1924	24	27	26	21	27	....*
	1925	25	25	23	19	25	54
Radiobacter group 10,000 per gram soil.....	1924	35	40	35	25	30	....
	1925	34	42	33	15	24	45
Actinomycetes, millions per gram soil.....	1924	5.6	5.8	5.5	7.2	6.1	....
	1925	6.5	7.0	7.0	7.6	7.7	9.7
Fungi; 10,000 per gram soil. ....	1924	9	14	11	10	16	....
	1925	11	13	11	12	14	17
Mgm. N assimilated in mannite solution.....	1924	7.6	6.7	6.7	2.9	3.3	....
	1925	6.3	6.0	5.6	3.7	3.2	4.4
Mgm. N nitrified in $(\text{NH}_4)_2\text{HPO}_4$ solution.....	1924	4.4	4.7	5.0	3.2	4.3	....
	1925	5.1	5.6	5.7	3.3	4.0	5.7
Nitrate N in soil, parts per million ...	1924	....*	....	....	....	....	....
	1925	3.0	11.0	7.9	0.52	1.20	13.6

\* No determinations made.

vetch) than under cereals, this does not indicate a strong nitrate assimilation by soil bacteria.

The results of nitrification experiments in ammonium phosphate solution, recorded in table 9, indicate a stronger nitrification under and after legumes, confirming and supplementing the nitrate determinations made directly with the different soils. The nitrogen assimilation in mannite solution, on the other hand, has not proved to be superior under legumes. It must be kept in mind, however, that the conditions of this experiment are favorable to Azotobacter,



as well as to *Amylobacter*, but are not especially suited to the requirements of other nitrogen-fixing bacteria, such as *B. radiobacter*. Therefore, the only conclusion justified is that *Azotobacter* and *Amylobacter* were no more stimulated in their development by the growth of legumes than by that of non-legumes.

Alternating with the experiments made with greenhouse soil, analogous tests were made with samples taken from part of the field plots during the growing season. In table 10 the averages are given as calculated from 4 and 5 successive tests, made from April to August, 1924 and from March to July, 1925, respectively. The consistency of the results in both years is quite marked, and it is likewise evident that the data obtained under field conditions agree well with those secured in the greenhouse. Since the soil of field I is somewhat different from that of field II, only the effects of the crops grown on the same field can be compared unrestrictedly. The much stronger nitrogen fixation in mannite solution inoculated with soil from field I is of special interest in view of its increased productivity discussed before.

Under and after legumes again the total bacterial counts are somewhat larger than under cereals and the number for the *Radiobacter* group is markedly higher under vetch and cowpeas, but not under soybeans. The actinomycetes and fungi show slight changes again in favor of the legumes, as do the nitrification and to a still larger extent the nitrate content of the soil.

Late in the fall of 1924 the total number of bacteria under cowpeas reached a maximum of 47.5 million, and the *Radiobacter* group 1,400,000 colonies per gram soil. At the same time the plowed wheat stubble showed a total count of 44.5 millions, and 470,000 *Radiobacter* colonies per gram soil. The tilth of the soil was very satisfactory at this time.

The bacterial counts obtained with samples taken from a strip of original sod that was left untouched between fields I and II when the field experiments were started in 1914, show how uniform the results of such tests will be if the environmental conditions are uniform and a faultless technique is carefully applied. Throughout the year 1924, the following total counts (millions per gram soil) were secured on soil extract agar, as the average of two soil samples taken under the sod 4 inches below the surface: April, 21.8; May, 19.0; July, 20.0; August, 22.8; October, 20.1; November (2 tests), 21.8 and 17.0.

These widely fluctuating results, which are not unusual (32), should not be ascribed to erratic daily or hourly variations in the bacterial flora of the soil.

#### DISCUSSION

The experiments reported have shown conclusively that the beneficial after-effect exerted by legumes harvested for hay is to a considerable extent due to favorable changes in the microflora of the soil, which are still marked, and are even increasing a few weeks after the surface growth of the legumes has been removed. Several months later, and especially after the soil has dried out thoroughly, this beneficial effect upon the succeeding crop is no longer

noticeable. The increases in the succeeding crops caused by this after-effect of harvested legumes are frequently larger than those caused by legumes used as green manures. It is probable that in field tests the crop increases ascribed to green manuring are in fact more frequently due to the special after-effect of the growing legumes than to their manuring effect after having been plowed under where they had been grown.

These findings are supported by practical experience as well as by earlier experimental results. Tests made at the Arkansas Station more than twenty years ago (29, 30, 31) showed that the increases in the winter wheat crop were larger when the legumes were removed than when they were turned under, but that with spring oats, opposite results were obtained. Analogous findings were recorded at the same time in Alabama (6) where they were accepted as "unexpected." Again winter wheat and winter oats were more benefited by the legume stubble than by the legume as green manure, whereas with the crops planted in spring (cotton, corn, sorghum) opposite results were obtained. In a cowpea-wheat rotation tested at the Tennessee Station (26) practically the same wheat crop was raised whether the surface growth of the cowpeas was removed or whether it was turned under. Experiments made in Virginia (1) with clover, soybeans, buckwheat, and rye on corn gave likewise approximately the same results with the stubble as with the whole plant turned under. In cylinder experiments conducted at the New Jersey Station (21) the nitrogen returns were not much higher if cowpeas, crimson clover, or winter vetch were turned under where they had been grown, than in those cases where their surface growth was removed and tested separately.

Field peas and vetches were found by J. G. Lipman (20) to exert a more beneficial effect upon non-legumes than cowpeas and soybeans, and the same relation was noticeable in our experiments. Practical experience (27) as well as the pot tests reported, shows that soybeans are regularly less beneficial to the succeeding crop than are cowpeas. The fact that not more than two per cent of the legumes grown in America are being used as green manures furnishes additional proof that it is the beneficial after-effect of the growing legumes which is of greatest importance.

The detrimental effect sometimes noticed with heavy green manuring, but more frequently overlooked because of the counter-action due to the beneficial after-effect of the growing legumes, may have several causes. If relatively large quantities of green matter are used, as is often the case in pot tests (36), acid and ammonia production, heavy mold growth, and other factors which are hardly of any importance under field conditions, may prove detrimental. Nitrate assimilation is probably of greatest importance in the field, since nitrification is enhanced under and after legumes, whereas the addition of fresh organic matter tends to stimulate the opposite reaction. To use leguminous green manure on a field other than where it was grown will give better results, as a rule. Mulching deserves special attention in this connection (12).

Generally preferable, however, is the feeding of the legumes and the application of the stable manure obtained from them, as is demonstrated by the

increased crops and the unimpaired nitrogen content of the plots so treated in our field experiments. It is true that the humus and nitrogen content of a soil may remain constant for some time, even if only the crop residues are being left (2). As a rule, and especially after the original humus content of a soil is as far reduced as is the case in many fields of the eastern and southern part of the United States, the use of organic manures is essential to restore and maintain the vanishing natural soil productivity. A comparatively small amount of stable manure, however, is sufficient for this purpose, and as shown by the field experiments an unusually high efficiency may be secured in this case.

The low nitrogen efficiency of mineral nitrogenous fertilizer recorded in the second half of the crop rotation experiment in the field is undoubtedly due to lack of humus, causing a deterioration of the soil texture and an insufficient production of carbon dioxide. Equally incomplete nitrogen returns have been noted in other tests made with nitrate under similar conditions (22). As in the field experiments discussed, the after-effect of legumes cut for hay was equal or superior to the application of nitrate, whereas ripe legumes again proved inferior (11).

It has been stated occasionally that "very revolutionary practices" would have to be instituted in this country in order to retain proper relations between food supply and increasing population (8). The experiments discussed, however, show that it is quite feasible to double and treble the grain crops within a few years by growing cereals and legumes best adapted to soil and climate in suitable rotations, and there is no doubt that at least in all regions where legumes can be grown successfully, their proper use will assure much larger grain crops than are needed at the present and in the near future. Wherever the natural soil productivity is now rather low, because of a long continued growth of grain crops, it seems highly advisable from an economic standpoint to raise the same quantities of cereals on a smaller tilled area by adopting more efficient crop rotations, and to increase at the same time the natural fertility of the remaining area by using it for pasturage or for producing forage rich in legumes. Any future emergency would then easily be overcome by returning this enriched land to the production of heavier grain crops.

#### SUMMARY

Field, greenhouse, and laboratory experiments on the beneficial influence exerted by growing legumes upon succeeding crops have furnished the following results:

1. Comparative field experiments showed that the increases in the crops after legumes were almost or quite as high when the legumes were harvested as when they were plowed under as green manures. The maximum increase observed in pot tests was close to 100 per cent by green manuring, but over 200 per cent by the growth of legumes harvested for hay.

Several reasons of this beneficial after-effect were discussed and investigated. In the field the suppression of noxious weeds is of great value, whereas not much importance can be attached to the amount of nitrogen contained in stubble and roots. Probably of greatest importance is the stimulating effect exerted by the growing legumes upon the bacterial activities in the soil.

2. The stimulation of bacterial activities by growing legumes was evidenced by marked increases in the total number of soil organisms enumerated on soil extract agar, by rapid multiplication of *B. radiobacter* and related forms, and by greatly intensified nitrification, Actinomycetes and fungi became likewise more numerous under the influence of legumes, though to a much smaller extent than the bacteria. Numbers of *B. radiobacter* as high as under legumes were observed only once in a plowed wheat stubble after this soil had attained its perfect tilth in fall. The favorable change in the soil flora persisted and became even more pronounced during the next two or three months after the legumes had been harvested. Longer intervals, however, and especially a thorough drying of the soil caused a more or less complete disappearance of these beneficial changes.

3. These observations explain the great benefit to the soil productivity of replanting soon with cereals or hoed crops a field upon which legumes have been grown. Naturally, a marked after-effect is dependent on the proper selection of the legumes used in the crop rotation; these will be different according to soil and climate. On the experimental field only three of a large number of legumes proved entirely satisfactory, viz., cowpeas and soybeans as summer crops, and hairy vetch as a winter cover crop. These legumes, grown every fourth year on the field, assimilated from the air approximately 80 pounds nitrogen per acre and year and exerted, after their surface growth had been removed, an after-effect equivalent to that obtained by the application of 30 pounds nitrate nitrogen per acre and year.

4. Greenhouse tests showed that field peas, hairy vetch, and cowpeas were of greatest benefit to the microflora of the soil and to the succeeding grain crops. Soybeans, on the other hand, were hardly of any influence in this direction, although they gave quite satisfactory results when used as green manure. The growing of two or three crops of field peas, hairy vetch, or cowpeas for hay, alternating with five crops of corn increased the latter to such an extent that their total weight exceeded that of seven crops of corn and small grains raised without legumes.

5. In a heavy clay soil poor in humus, considerable losses of soil nitrogen occurred under 29 crops of non-legumes, whereas conspicuous gains in nitrogen, equivalent to one-third of the total nitrogen originally present in this soil, were secured when legumes were grown a few times between the non-legumes. No losses in nitrogen occurred in a soil of better chemical and physical quality. Despite greater natural productivity, this soil gave persistently lower counts in soil organisms than were obtained with the poorer soil.

6. A low humus content in field soil caused a rather low efficiency of nitrogen applied as nitrate or ammonium sulfate. Only 40 per cent was recovered in the crops. On the other hand, stable manure used in relatively small amounts (15 tons per acre in an eight-year rotation) showed a nitrogen efficiency of 75 per cent. Probably in addition to the improvement of the physical structure of the soil, the increased development of carbon dioxide from organic manures is of considerable importance under such conditions.

7. Long continued greenhouse tests on the effect of different crop successions are an excellent means of securing more definite and more thorough information upon this important subject. The precautions to be observed in such experiments have been discussed, and it has been shown that very reliable data can be secured, if these tests are made on a broad basis and for a sufficient length of time.

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## BOOK REVIEW<sup>1</sup>

*Handbuch der biophysikalischen und biochemischen Durchforschung des Bodens. (Handbook of Biophysical and Biochemical Soil Investigations.)* 1926.

STOKLASA, J. AND DOERELL, E. G. (Pp. xv + 812, fig. 91. Paul Parey, Berlin.)

The senior author of this book, Professor of the Technological Institute and State Experiment Station at Prague, Czechoslovakia, has produced, with the collaboration of the junior author, an exceptionally fine contribution to the subject of soil science. The senior author, who recently celebrated the fortieth anniversary of his scientific activities, himself took a prominent part in various phases of the development of our knowledge of microbiological processes in the soil and their bearing upon plant growth. The book will be of great value to all those who are interested in the soil as a medium for the growth of higher plants, and especially as a guide to the teacher and investigator. The authors succeeded in covering the literature of the subject quite thoroughly, largely avoiding the pitfalls of many other handbooks that frequently become merely an encyclopedic enumeration of literary contributions, without any critical interpretation.

The volume is made up of the following sections: Introductory. *Biophysical and biochemical investigations of the soil.* Methods of soil investigation. Mechanical soil analysis. Determination of water-holding capacity of the soil. Soil atmosphere. Chemical analysis of the soil. Adsorptive power of the soil. Determination of electrical conductivity of the soil. Soil reaction. Methods of determination of soil acidity. Gas-chain method of Michaelis. Field method of electrometric determination of soil acidity after M. Trénel. Colorimetric methods. Determination of hydrogen-ion concentration of soil by a modified method of Michaelis. Other methods for determination of soil reaction. Exchange acidity. Critical remarks concerning the soil reaction and its determination electrometrically and colorimetrically. Special chemical investigation of the soil. Determination of nutrients in soil extracts. Determination of radio-activity in soil and in soil air. On the influence of natural radio-activity of minerals and rocks upon the germination and development of plants. Methods of determination of radioactivity in soil and in soil air. *Methods of biological investigation of soil.* General considerations concerning the microorganisms present in the soil. The bacteria of the rhizosphere. Methods of investigation of the edaphon. Isolation of the edaphon population and the biochemical

<sup>1</sup>This review was prepared by Dr. Selman A. Waksman, of the New Jersey Agricultural Experiment Station.



characterization of individual geobiont groups. Bacteria that participate in the cycle of nitrogen in nature. Protein synthesis in the soil. Methanecomposing bacteria. Hydrogen-oxidizing bacteria. Sulfur bacteria. Desulfurization in soil. Iron bacteria. Actinomyces. Fungi. Algae. Protozoa. Determination of excretory products in bacterial respiration. Biological absorption. Biochemical methods of determination of phosphoric acid and potash present in the soil in an assimilable form. Soil respiration. Carbon dioxide as an index of soil processes. Methods of determination of carbon dioxide evolution from soil. Experiments concerning the utilization of various organic substances by heterotrophic organisms as sources of carbon. Influence of the chemical nature of organic substances upon respiration processes in the soil. Decomposition of celluloses in the soil. Oxidation of organic nitrogenous compounds in the soil. Respiration intensity of microorganisms (auto- and heterotrophic) in various treated and untreated soils. Respiration of forest soils. Composition of drainage waters as an index of biochemical processes in the soil. On the influence of manure upon the mechanism of respiration in the soil. On the influence of radioactivity upon the dissimilation processes of microorganisms in the soils. Conclusions.

On the whole, the various phases of biochemical processes in the soil are extensively and adequately treated. The author emphasizes particularly the results of his own investigations during the long period of his scientific activities; however, other methods and results are considered sufficiently, so that the book has not been made too one-sided.

It is easy to find faults in a book of this nature, where practically virgin ground is covered and where an attempt is made to review a most extensive literature published in various languages, touching upon a number of phases of as complicated a subject as soil science. For example, one might say that altogether too much space is devoted to the determination of soil reaction (100 pages) and to the radioactivity of soil (46 pages) and that rather insufficient consideration is given to certain important groups of soil organisms—especially to those non-bacterial in nature; namely, the fungi, actinomyces and protozoa—and to certain microbiological activities, such as the nature of partial sterilization of soil. In speaking of soil adsorption and exchange of bases, the authors consider only the work of Hissink, without even mentioning the important contributions to this subject by Gedroiz. Although an excellent discussion is given of the methods of determination of “humus” and “humic acids,” nothing is presented to indicate the origin of these organic complexes in the soil and the rôle of microorganisms in these processes. Too much emphasis is laid upon bacterial fertilizers, consisting of composts of peat and phosphoric acid inoculated with bacteria from the root system (rhizosphere) of cultivated plants; this process is even emphasized as a triumph of modern biochemical technic—unfortunately we have altogether too much evidence to indicate that the numerous “all-crop inoculants” have proved to be largely failures. The soil harbors sufficient organisms capable of pro-

ducing abundant transformations, if conditions are favorable to their development. If one is justified in inoculating a freshly drained and limed peat soil with good garden soil, or a normal field soil with certain specific organisms such as the nodule bacteria, the use of all-crop inoculants is more than questionable. One would also like to take exception to the statement (p. 396) that the use of Alinit was the stimulus to the development of the whole science of soil bacteriology—perhaps the discouragement experienced by many workers in the field is due to that kind of a stimulus. The reviewer does not want to give, however, too much consideration to the defects which tend to detract from the otherwise excellent qualities of the book. The literature is covered thoroughly; here again any disagreements that one might present would be rather in connection with a difference in interpretation of the results.

The book is well printed and has a most extensive bibliography, consisting of thousands of references, placed very conveniently at the bottom of the respective pages. Detailed author and subject indices are appended. Some misprints have been noted. Dr. H. J. Conn (p. 424) of the New York Experiment Station is referred to as the Swiss bacteriologist probably because of the fact that the Experiment Station is at Geneva, N. Y.



# ON THE ORIGIN AND NATURE OF THE SOIL ORGANIC MATTER OR SOIL "HUMUS": IV. THE DECOMPOSITION OF THE VARIOUS INGREDIENTS OF STRAW AND OF ALFALFA MEAL BY MIXED AND PURE CULTURES OF MICROÖRGANISMS<sup>1</sup>

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It has been demonstrated that when straw is acted upon by pure cultures of fungi or bacteria in soil or in sand media, the sugars, hemicelluloses, and celluloses are readily decomposed, but the lignins are not acted upon. As a matter of fact the presence of the lignins even prevents, to a certain extent, the rapid decomposition of the celluloses with which they are combined. These lignins accumulate in the soil, especially in the absence of, or under conditions which do not favor the development of organisms capable of decomposing them. This accumulation contributes to the formation of the soil "humus." It remains to be seen whether a mixed soil flora will behave in normal soils in a similar way toward the lignins.

For this purpose, barley straw and alfalfa meal (green alfalfa, dried and ground) were treated in a manner outlined previously, for the purpose of removing consecutively different constituents or groups of the natural organic matter. Ether was used this time to remove the fats and waxes; this was followed by 95 per cent alcohol, then by cold water for 24 hours. The material from which these fractions were removed was then treated with 5 per cent NaOH solution for 30 minutes at 15 pounds pressure. The liquid was filtered off. The residue was washed with water and acetic acid and then treated with 2 per cent  $\text{H}_2\text{SO}_4$  solution for 2 hours at boiling temperature. At every step of the process, portions were set aside and used afterward in the study of decomposition. Pure lignin was prepared for the following studies by a modification of the Willstätter method, suggested by Schwalbe (4). This consists in treating 3-gm. portions of the organic materials from which the ether, alcohol, and water-soluble portions have been removed, with a mixture of 72 per cent  $\text{H}_2\text{SO}_4$  (60 cc.) and 15 cc. of 1:1 HCl (18 per cent), in a glass stoppered bottle, which is kept immersed in cold water during the reaction. After the reaction is completed, the mixture is transferred to a flask with about 500 cc. of distilled water and boiled for 1 hour. The lignin is then

<sup>1</sup> Paper No. 279 of the Journal Series, New Jersey Agricultural Experiment Stations, Department of Soil Chemistry and Bacteriology.

filtered through paper and washed until all traces of the inorganic acids have been removed.

An analysis of the two natural organic substances used is given in table 1.

One-gram portions of the different dry preparations were then added, in duplicates, to 100-gm. portions of sieved fresh soil, placed in 300-cc. round bottom flasks. Five-cubic centimeter portions of a nutrient solution containing 2 gm.  $(\text{NH}_4)_2\text{SO}_4$  and 4 gm.  $\text{K}_2\text{HPO}_4$  in 100 cc. of water, were added to each flask. The soil was then well mixed, brought to optimum moisture with distilled water and connected with the respirator used in the study of the evolution of  $\text{CO}_2$ ; the whole apparatus was kept in the thermostat at 27 to 28°C. After 35 days incubation, in one case, and 32 days in another, the

TABLE 1  
*Composition of barley straw and alfalfa meal\**

PREPARATION	FRACTION NUMBER	STRAW		ALFALFA MEAL	
		Yield	Nitrogen extracted from 10 gm.	Yield	Nitrogen extracted from 10 gm.
		<i>per cent</i>	<i>mgm.</i>	<i>per cent</i>	<i>mgm.</i>
Original material, moisture content.....	I	9.38	....	12.30	....
Ether-soluble substances.....	II	1.35	....	1.15	....
Alcohol-soluble substances.....	III	3.14	....	4.64	....
Loss on extraction with cold water for 24 hours.....	IV	7.46	8.8	15.41	33.0
Loss on extraction with 5 per cent NaOH at 15 pounds pressure.....	V	31.51	31.4	31.70	132.5
Precipitate of NaOH extract with HCl.....	VIII	19.06	....	10.98	....
Lignin, by method of Schwalbe†.....	VII	15.58	....	14.67	....
Loss on treatment with 2 per cent $\text{H}_2\text{SO}_4$ .....	VI	10.90	....	8.96	....
Residual material (cellulose) after treatment with 2 per cent $\text{H}_2\text{SO}_4$ .....	VI	36.22	....	25.84	....

\* Total nitrogen content of the straw 0.41 per cent, of alfalfa meal 2.44 per cent.

† Determination made on preparation, after ether, alcohol, and water-soluble fractions have been removed, but calculated on basis of original moisture-free material.

soils were removed and aliquot portions used for the determination of ammonia (by replacement with KCl solution, then distilling with  $\text{MgO}$ ), of nitrates (by the phenoldisulfonic acid method), and of "humus" (by the method reported above). The results are given in tables 2 and 3.

The removal of various fractions from natural organic materials, such as barley straw and alfalfa meal, by solvents, influences materially both the rate and the amount of decomposition of the organic matter, within a definite period of time. Although the separation of the various ingredients was probably in some cases incomplete, the results point definitely to the fact that natural organic matter contains ingredients which are acted upon more readily by the mixed soil flora and fauna, ingredients which are decomposed more

TABLE 2  
The decomposition of different fractions of barley straw by the mixed soil flora, in untreated soil\*

PREPARATION	FRACTION NUMBER	CARBON EVOLVED AS CO <sub>2</sub> IN						TOTAL CO <sub>2</sub>	CONTROL SOIL SUBTRACTED	RESIDUAL AVAILABLE NITROGEN NH <sub>4</sub> - N + NO <sub>3</sub> - N IN 100 Gm. OF SOIL	AMOUNT OF NITROGEN USED UP	HUMUS, α-FRAC-TION IN 100 Gm. OF SOIL
		2 days	5 days	8 days	14 days	18 days	23 days	35 days				
Control soil.....	I	5.8	4.8	3.4	6.0	5.8	5.2	13.8	.....	22.9	....	553
Untreated straw.....	II	17.7	29.1	21.5	36.9	22.0	14.0	27.4	123.8	16.0	6.9	673
Ether fraction removed.....	III	18.5	31.6	21.5	41.1	20.3	14.4	27.0	129.6	15.1	7.8	733
Ether and alcohol fractions removed.....	IV	16.3	30.0	22.3	36.6	20.6	15.3	29.2	125.6	15.1	7.8	707
Ether, alcohol, and water-soluble fractions removed.....	V	10.7	28.4	21.9	37.5	23.8	14.9	30.5	122.9	15.7	7.2	683
Ether, alcohol, water, and alkali-soluble fractions removed.....	VI	11.4	33.9	30.1	43.0	30.9	20.0	35.8	160.3	13.5	9.4	610
Residual cellulose.....	VII	6.4	21.2	29.3	58.5	35.1	22.6	39.5	167.8	11.3	11.6	513
Alkaline extract precipitated with HCl	VIII	15.1	21.0	14.3	19.3	9.4	9.6	20.8	64.7	19.1	3.8	1,030
Lignin, Schwalbe method.....	IX	5.5	4.9	6.0	7.5	5.0	4.7	14.2	3.0	22.7	0.2	1,242

\* 1 gm. portions of different preparations used in 100 gm. of soil, 20.5 mgm. of nitrogen added to each soil portion, in the form of ammonium sulfate.

TABLE 3  
*The decomposition of different fractions of alfalfa meal by the mixed soil flora, in untreated soil\**

PREPARATION	FRACTION NUMBER	CARBON EVOLVED AS CO <sub>2</sub> IN							TOTAL CO <sub>2</sub>	CONTROL SOIL SUBTRACTED	RESIDUAL AVAILABLE NITROGEN NH <sub>4</sub> -N + NO <sub>3</sub> -N IN 100 GIL OF SOIL	HUMUS, C <sub>2</sub> FRAC-TION IN 100 GIL OF SOIL
		2 days	4 days	7 days	11 days	16 days	19 days	32 days				
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
Control soil.....	I	5.0	....	7.6	4.8	4.2	4.8	15.2	41.6	....	24.05	612
Untreated alfalfa meal.....	II	28.8	35.6	22.0	17.9	21.0	19.9	22.4	167.6	126.0	26.80	725
Ether fraction removed.....	III	28.4	41.8	21.7	16.3	18.4	15.1	27.1	168.8	127.2	28.30	686
Ether and alcohol fractions removed.....	IV	29.6	31.1	19.2	17.9	20.7	13.7	23.8	156.0	114.4	23.80	680
Ether, alcohol, and water-soluble fractions removed.....	V	15.4	20.6	18.9	22.0	20.7	14.8	21.2	133.6	92.0	21.30	709
Ether, alkali, water, and alkali-soluble fractions removed.....	VI	14.1	26.4	19.0	17.5	24.3	16.6	39.1	157.0	115.4	14.50	653
Residual cellulose.....	VII	7.3	17.0	24.9	20.0	26.9	19.0	33.7	148.8	107.2	16.20	667
Lignin by Schwalbe method.....		4.8	....	7.0	5.3	4.6	6.4	15.5	43.6	2.0	24.30	1,237

\* 1 gm. portions of different preparations used in 100 gm. of soil, 20.5 mgm. of nitrogen added to each soil portion, in the form of ammonium sulfate.

slowly and ingredients that are left undecomposed, at least within the short period of time used in the experiment. The results tend also to throw light upon the influence of some constituents (fats, lignins) upon the decomposition of others.

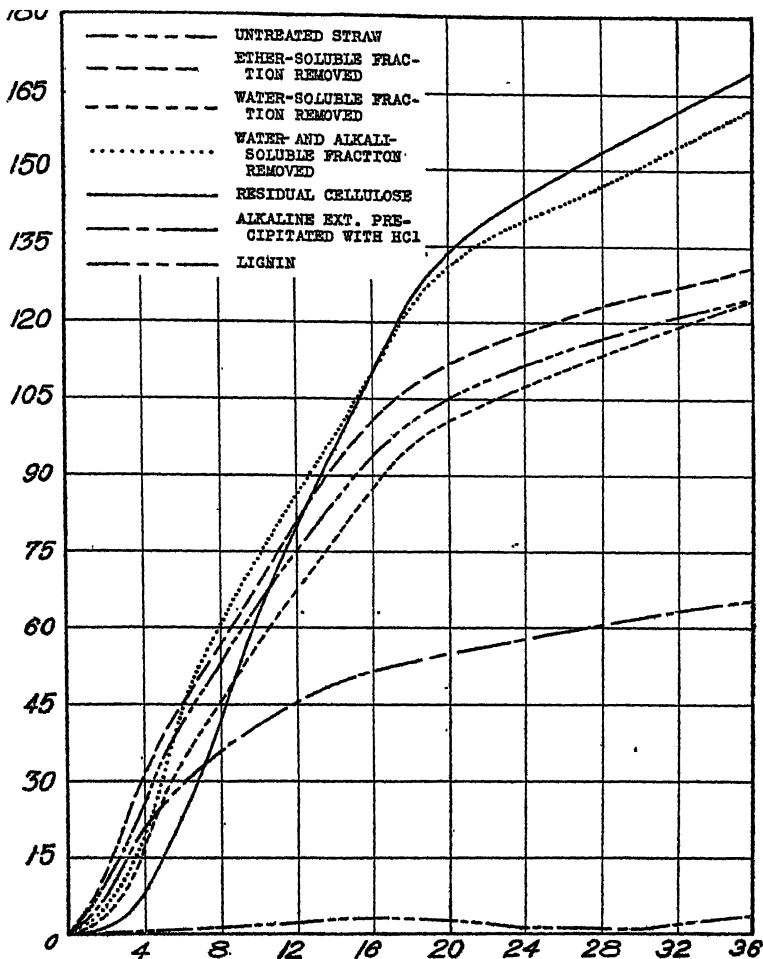


FIG. 1. COURSE OF DECOMPOSITION OF CEREAL STRAW AND ITS VARIOUS FRACTIONS

The removal of the fats and waxes from the straw hastened somewhat its decomposition, especially at the early stages. The substances removed by ether are not readily acted upon, and they seem to prevent even to a very limited extent the decomposition of the natural organic matter. The same is true, perhaps only to a very limited extent, of alfalfa meal. The treatment of straw with ether and then with 95 per cent alcohol resulted in the



removal of about 4 per cent dry matter. The treated material decomposed at first a trifle more slowly, then only a trifle more quickly than the untreated straw. The favorable effect of the preliminary ether treatment seemed to have been balanced by the unfavorable effect of the alcohol treatment, the latter resulting in the removal of some sugars and amino compounds—sub-

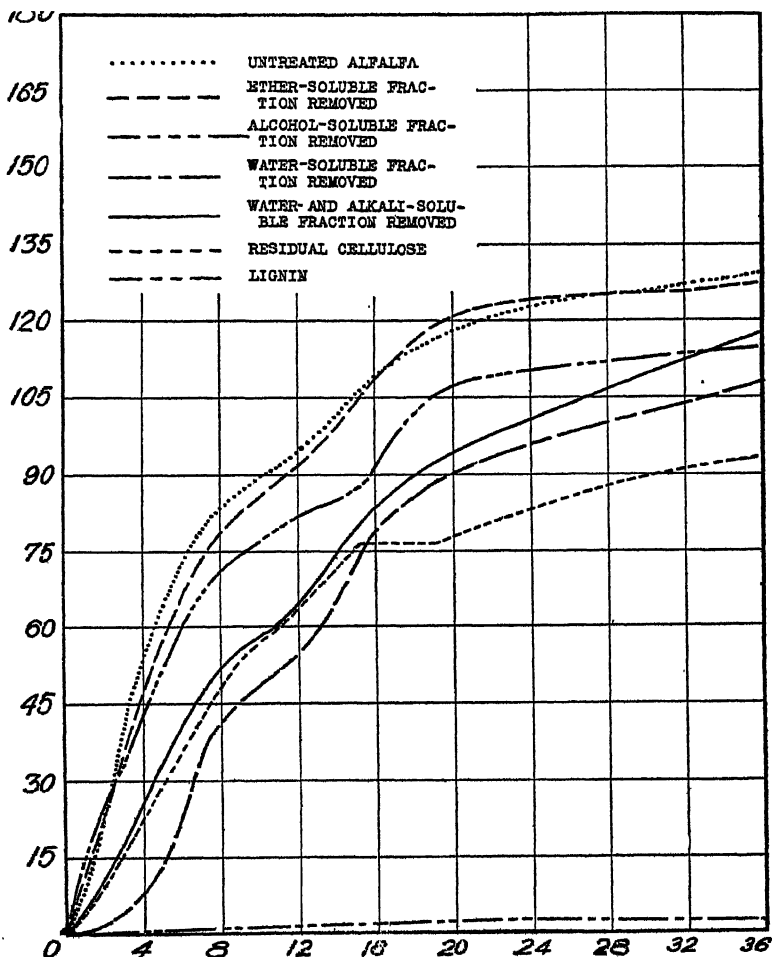


FIG. 2. COURSE OF DECOMPOSITION OF ALFALFA MEAL AND ITS VARIOUS FRACTIONS

stances which are the first to be acted upon when natural organic matter is added to the soil. This is especially marked in the case of the alfalfa meal, which was treated with ether and with alcohol. The preliminary ether treatment did not seem to have a markedly favorable effect; the alcohol, however, removed a considerable amount of reducing sugars and amino acids, namely

the ingredients most readily acted upon. This resulted in a retardation in the decomposition of the organic matter and in a reduction of about 10 per cent in the total amount of  $\text{CO}_2$  produced in 35 days from 1 gm. of the preparation.

Since straw contains a comparatively small amount of water-soluble substances (7.46 per cent), one would expect that the removal of these would affect the decomposition of the straw only to a limited extent. The alfalfa meal, however, was found to contain a considerable amount (15.41 per cent) of water-soluble substances and one would expect that their removal would materially influence the rate of decomposition of this organic substance. This was actually found to be the case, especially in the early periods of incubation, since the water-soluble constituents of the natural organic matter are the first to be attacked and decomposed. The presence of a considerable quantity of water-soluble constituents (sugar, starches, amino acids, soluble proteins) in alfalfa meal accounts for the very rapid rate with which this and other legumes are decomposed during the early periods of incubation in the soil, as compared with straw and with cellulose (2, 5).

The removal of 15.41 per cent of water-soluble constituents from the alfalfa reduced considerably its rate of decomposition, as indicated by the evolution of carbon dioxide. The untreated alfalfa gave off 29 and 36 mgm. in 2 and 4 days; the removal of the water-soluble fractions reduced the amount of  $\text{CO}_2$  given off (as carbon) to 15.4 and 20.6 mgm. respectively, a rate of decomposition just a trifle lower than untreated straw. In other words, the removal of the water-soluble constituents changes an organic substance with a rapid rate of decomposition, into an organic substance with a slow rate of decomposition. The reason for the more rapid decomposition of alfalfa meal and of other green manures than of straw and of similar organic residues is thus to be looked for in the presence of an abundance of water-soluble constituents in the former.

Alkalies remove from natural organic materials a considerable part of the lignins and a part of the pentosans (and other hemicelluloses). The treatment with hot 5 per cent sodium hydroxide under pressure, of straw and alfalfa from which the ether-, alcohol-, and water-soluble fractions have been removed, did not increase the actual concentration of the materials which decompose rapidly. This was indicated by the fact that the rate of decomposition of the substances left after the alkali treatment, was not at first any greater than the decomposition of those from which the alkali-soluble substances had not been removed. The treatment, however, had a definite influence upon the decomposition of the residual substances, as seen by the fact that the rate of decomposition rapidly increases even within the first few days, soon greatly exceeding the decomposition of the material which has not been treated with hot alkali solution. This is especially true in the case of the straw, where the removal of the alkali-soluble materials increased the total amount of decomposition, in the experiment under consideration, by 33 per

cent. This explains the common practice (3, 1) of boiling straw with alkalis to increase its digestibility. The removal of all or even of only a part of the lignins favors considerably the digestibility of the celluloses by microorganisms. The results of the investigations of the decomposition of straw by pure cultures of microorganisms, reported previously, and by the mixed soil flora are thus found to give comparable results, as far as the influence of the lignins upon the decomposition of celluloses and hemicelluloses in the soil is concerned.

When the organic materials from which the alkali-soluble substances have been removed, are treated further with dilute acids, the decomposition of the residual celluloses is not affected to any considerable extent, for this treatment removes the hemicelluloses (including the pentosans) which have not been removed by the alkali treatment. Both hemicelluloses and celluloses decompose in the soil at about an equal rate and are controlled alike by conditions.

The decomposition of the lignin preparations is especially interesting, since very little is known concerning the decomposition of lignins in the soil. It has been shown previously that lignin prepared directly from organic materials cannot be decomposed by pure cultures of cellulose-decomposing fungi and bacteria. The preparation obtained by precipitating the alkali extract of the soil with hydrochloric acid is not pure lignin but is rich in hemicelluloses, the presence of which accounts for the decomposition of that material in the soil. In these experiments, the extract obtained by treating straw, from which the ether-, alcohol-, and water-soluble constituents have been removed, with 5 per cent hot NaOH under pressure, then precipitating the extract with hydrochloric acid, was found to consist of almost 50 per cent hemicelluloses and about 50 per cent lignin. When this preparation, washed and dried, was added to the soil, it was found to decompose readily at first, then more slowly; the actual amount decomposed was about one-half that of the untreated straw, as shown by the evolution of  $\text{CO}_2$ . The fact that also about one-half as much nitrogen was used as in the case of the whole straw, shows that there is a definite relation between the  $\text{CO}_2$  evolved and the nitrogen assimilated in the whole straw and in its various constituents and also that only about one-half of this preparation was undergoing decomposition. The fact that the other half did not undergo decomposition but went to increase the amount of soil organic matter that does not decompose readily, is borne out by the figures in the column of soil "humus."

The addition to the soil of 1 gm. of lignin, prepared according to the method of Schwalbe, did not increase the rate of evolution of  $\text{CO}_2$  from the soil itself. Both lignin from straw and lignin from alfalfa meal gave an increase of only 2 or 3 mgm. of carbon as  $\text{CO}_2$  for periods of 35 and 32 days; this quantity of  $\text{CO}_2$  lies within the experimental error of the determinations. In other words lignin obtained from straw, and lignin obtained from alfalfa meal practically did not decompose at all in the soil within a period of 35 days. This bears out

the results of various investigators including those of the senior author reported previously that *lignin forms one of the few constituents of the natural organic matter which does not decompose in the soil but accumulates there, thus contributing to the "humus" of the soil.* This is also demonstrated by the actual analyses of the "humus" in the soil.

The amount of available nitrogen assimilated by the organisms in the process of decomposition of natural organic materials is a very good index of the amount of decomposition that has taken place. A definite parallelism between the amount of nitrogen assimilated by the organisms in the soil and the quantity of cellulose decomposed, reported previously, is also well illustrated in table 2, where the amount of nitrogen used up by the microorganism decomposing the straw or its various constituents is exactly parallel to the amount of  $\text{CO}_2$  produced, the ratio being in practically all instances 16 or 18 to 1; in other words, 16 to 18 mgm. of carbon is liberated as  $\text{CO}_2$  by a mixed soil from straw or its various constituents for every unit of nitrogen assimilated by the microorganisms and changed from an inorganic (as ammonium sulfate) into an organic form. When these results are compared with those previously reported, it is found that the ratio of  $\text{CO}_2$  evolution to nitrogen assimilation is 7.0 to 8.0 for pure cultures of fungi, 12.5 for pure cultures of bacteria, and 16 to 18 for the mixed soil flora. The smaller amount of nitrogen used by the mixed soil flora is due largely to the secondary decomposition processes, whereby the protoplasm synthesized by one group of organisms, in the process of decomposition of the carbonaceous materials, is again decomposed by other members of the soil flora, with the result that the nitrogen is again liberated as ammonia and can enter again into circulation. The ratio between the carbon (or energy) decomposed or liberated as  $\text{CO}_2$  and the nitrogen assimilated, or protoplasm synthesized, is very definite for the different straw preparations, but varies with the nature of the organisms carrying out the decomposition. This indicates definitely that the nature of decomposition of organic matter, the amount of  $\text{CO}_2$  liberated, the amount of nitrogen required for its decomposition, and, probably to a large extent, the "humus" formed, depend largely upon the organisms carrying out the decomposition and upon the environmental conditions favoring the development of the particular organisms rather than upon the nature of organic matter added. Of course the rapidity of decomposition of the organic matter and the nitrogen liberated as ammonia depend upon the composition of the organic matter, which is modified by the nature of the plant and its stage of maturity.

When the data obtained from the decomposition of the various constituents of alfalfa meal are considered in this light, it is found that the decomposition of the whole alfalfa plant does not require any additional nitrogen, but that some is even liberated as ammonia and nitrate (2.75 mgm. nitrogen from 1 gm. of alfalfa meal in 35 days). The removal of the ether- and alcohol-soluble fractions reduced the amount of decomposition by 10 per cent, as pointed out

above, and reduced the nitrogen balance from a gain of 2.75 mgm. of nitrogen, in the form of ammonia and nitrate, to a loss of 0.25 mgm. The removal of the water-soluble constituents and especially of the alkali-soluble constituents, which included most of the proteins of the alfalfa meal, resulted in a preparation similar to straw not only in the decomposition rate but also in the amount of nitrogen required for this purpose. When the "cellulose" preparations of straw and alfalfa meal are compared, there is found in both cases a similar ratio between the carbon of the  $\text{CO}_2$  liberated and the nitrogen changed from an inorganic into an organic form, namely  $14.46 \left( \frac{167.8}{11.6} \right)$ , in the case of the straw preparation, and  $13.66 \left( \frac{107.2}{7.85} \right)$ , in the case of the alfalfa preparation. Differences in the rate of decomposition of various organic materials, as green manures at different stages of growth and residues of different plant and animal origin, can thus be readily explained by the qualitative and quantitative differences in the amount of the various constituents of the different plants, their solubility, etc.

The results of the "humus" determinations are very illuminating. Only the results of the  $\alpha$  fraction, or that part of "humus" which is soluble in alkalis (NaOH) and precipitated by acids (HCl) and which is equivalent to the so-called "humic acid," are reported here, since the same soil was used in all cases and the  $\beta$  fractions did not vary greatly. The straw was found to contain 15.5 per cent of lignin by the Schwalbe method and about 18 per cent by the Wilstätter method. By treating the straw with 5 per cent NaOH in the autoclave, only about two-thirds of the lignin is extracted. If the actual lignin content of the straw is assumed to be 18 per cent and that, by treatment with alkali and precipitation with HCl, only about two-thirds of this lignin can be obtained, the latter is quantitatively recovered in the "humus." The addition of 1 gm. of untreated straw to 100 gm. of soil resulted in an increase of 120 mgm. in the fraction of the "humus." When the straw was treated with ether, alcohol, and water, the increase in the humus was 180, 154, and 130 mgm. respectively. When the straw fraction, previously treated with alkali which resulted in the removal of most of the lignin, was added to the soil the increase in the "humus" content in the soil was only 60 mgm. When the preparation was also treated with dilute acids, there was no increase in the "humus" content. In other words, the decomposition of 1 gm. of straw preparation (celluloses and hemicelluloses) from which the lignin fraction had been removed led to an increase of only inappreciable amounts of "humus" in the soil, tending further to confirm the theory that lignin is the ingredient of straw and other natural organic materials which contributes to the soil "humus."

The greatest amount of humus was obtained from the soil receiving the lignin preparations—689 mgm. in the case of the lignin by the Schwalbe method and 447 mgm. in the case of lignin obtained by extraction with NaOH

and precipitation with HCl. When lignin obtained by the Schwalbe method is treated with NaOH and the extract precipitated with HCl, only 70 to 75 per cent of the lignin is recovered. In other words the same amount of lignin prepared after Schwalbe can be recovered from 1 gm. of preparation by direct treatment with NaOH and precipitation with HCl as was obtained from 1 gm. of the preparation which had been added to 100 gm. of soil and incubated under optimum conditions for 35 days. Thus the *evolution of CO<sub>2</sub> shows that lignin does not decompose readily in the soil; the determination of the "humus" content in the soil shows that this lignin is recovered quantitatively in the soil "humus."* In other words, lignin when introduced into the soil is "humus" and both terms are synonymous, to a certain extent.

The "humus" determinations of the soil to which the different fractions of the alfalfa meal were added gave very similar results, although varying somewhat in quantity. The actual increase in the amount of "humus" recovered from the addition of 1 gm. of lignin from alfalfa meal was 625 mgm.—very similar to the figure obtained for the lignin of the straw and by extracting lignin directly with alkali and reprecipitating the extract with acid. This is due to the fact that lignin dissolves very incompletely in alkalies under ordinary pressure. It is important to keep in mind, in this connection, that the actual "humus" extracted from the soil by treatment with alkalies, is never larger than 60 to 80 per cent of the total soil organic matter, the insoluble part frequently being referred to as "humin."

It is interesting in this connection that van Suchtelen (6) found that when natural organic substances are added to the soil, the monosaccharides and pentosans are decomposed first, followed by the celluloses, pectins, starches and proteins; a strongly resistant carbonaceous residue is left which is decomposed only very slowly. The results presented in this and in the previous paper tend to confirm this as well as the ideas of other workers (7) that lignins are very resistant to decomposition by microorganisms and that they are the mother substances of soil "humus."

#### SUMMARY

1. The removal of ether-soluble fractions from barley straw was found to hasten somewhat the decomposition of the straw, but the treatment was without influence upon the decomposition of alfalfa meal.
2. The removal of alcohol- and water-soluble substances was of little influence upon the decomposition of the straw materials but greatly reduced the rapidity of decomposition of alfalfa meal.
3. The removal of the lignins from straw and alfalfa meal hastened the rapidity and increased the amount of decomposition of the residual materials.
4. Lignins are not decomposed in the soil, at least within the experimental period of 32 to 35 days; if they are decomposed at all, the amount of decomposition is only insignificant in comparison with the decomposition of the other constituents of natural organic matter.

5. The lignin introduced into the soil was recovered practically quantitatively at the end of the incubation period as soil "humus," allowing for the imperfection of the method of extraction of the lignin and "humus" from the soil.

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## ERRATA

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*"Ion exchange in relation to soil acidity," by W. P. Kelley and S. M. Brown*

*Page 289, footnote 2, should read "The translation was made jointly by S. M. DuToit and R. V. Allison."*

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*Review of German literature on soil science and plant physiology in 1925*

*Pages 213-219. All references to Landw. Vers. Sta. volumes 53 and 54 should read volumes 103 and 104, respectively.*





# MICROBIOLOGICAL ANALYSIS OF SOILS AS AN INDEX OF SOIL FERTILITY: X. THE CATALYTIC POWER OF THE SOIL<sup>1</sup>

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## INTRODUCTORY

The catalytic power of the soil, or the ability of soils to decompose hydrogen peroxide with the liberation of molecular oxygen, has received considerably less attention than any of the other soil microbiological processes studied previously (37, 38). As a matter of fact, the catalytic action of the soil is frequently not considered a microbiological process, but is ascribed to the various inorganic soil constituents and to the soil colloids (10). The origin of the catalytic power of the soil, however, is considered by some investigators to be largely a result of microbiological activities. Not only is catalase contained in the bodies of many microorganisms developing in the soil, but this enzyme is present in the various vegetable manures and plant residues added to the soil. Certain mineral constituents of the soil also exert a definite catalytic action. The addition of manure to the soil leads to a rapid development of microorganisms; however, it is still questionable whether this is accompanied by an increase in the ability of the soil to liberate oxygen from hydrogen peroxide. In winter, both the development of microorganisms in the soil and its catalytic action diminish rapidly, whereas in the warmer months of April to June, both increase again. König (14) observed that soils in which the microorganisms have been killed by steam or antiseptics possess a much lower catalytic action than untreated soils. If the catalase found in different soils is largely of microbiological origin and indicates present or past activities of microorganisms, one is justified in considering the catalytic activity of soil from the point of view of microbiological activities, in an attempt to learn whether there is any correlation between microbiological activities and soil fertility and whether the former can serve as an index of the latter.

## HISTORICAL

The literature dealing with the subject of catalase has been reviewed in detail by Oppenheimer (25), Battelli and Stern (3), and Morgulis (22). It is sufficient to call attention here to those contributions which have a direct bearing upon the problem under consideration.

It has been known since the work of Thénard (35) that plant and vegetable tissues are

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capable of decomposing hydrogen peroxide into water and molecular oxygen. Berzelius (4) and Schoenbein (29a) seem to have been the first to mention that soils possess a catalytic power. Impressed by the fact that catalase is found abundantly in the cells of bacteria and fungi, which are of common occurrence in the soil, Loew (17) suspected that the soil should contain also an abundance of catalase. This was confirmed by actual observation. It was König and his associates (12-15), however, who carried out a systematic series of investigations on this subject, and who showed that soils differ greatly in their catalytic action and that this property is due largely to the enzyme catalase.

Loew (17) differentiated between two different catalases: ( $\alpha$ )  $\beta$ -catalase, soluble in cold water, and ( $\beta$ )  $\alpha$ -catalase, insoluble in cold water but extracted by treatment for 15 hours with 0.2 per cent sodium hydroxide solution or for 1 hour with a sodium carbonate solution, at 30°. An aqueous solution free from bacteria was found to lose the  $\beta$ -catalase rapidly, as a result of auto-oxidation or some intramolecular change. Nitrates seemed to depress materially the action of the enzyme, without injuring the enzyme itself. Potassium salts retarded the reaction more than sodium salts; sodium carbonate, however, markedly stimulated the reaction. Acids were generally found to be injurious to the enzyme.

According to Sørensen (31), catalase has its optimum at neutrality (pH 7.0). Michaelis and Pechstein (20), however, claimed that the action of catalase increases by increasing alkalinity up to pH 9.0; Sørensen's results were ascribed to the toxic action of phosphates, which were used as buffers, the former using acetates and carbonates as buffering agents. Both Bodansky (5) and Morgulis (21) noted that the optimum reaction of catalase is at pH 7.0 to 7.5, the action decreasing rapidly on the acid side of the optimum but not on the alkaline. Stapp (32) found the optimum for the action of bacterial catalase to be at pH 7.5 to 8, with a drop on both sides of the optimum. Different acids vary in their toxicity, as shown by the following series (29):  $\text{HNO}_3 > \text{H}_3\text{PO}_4 > \text{H}_2\text{SO}_4 > \text{HCl}$ . The injurious action of the anions depends largely on the reaction of the medium, being very pronounced at pH 3.0 to 5.0 and hardly noticeable at pH 7.3 to 8.0 (27). It is interesting to note that Bach and Oparin (1) found that the addition of lime to the soil increases greatly the catalytic action of germinating seed.

Heating a bacterial suspension at 80°C. for 15 minutes is sufficient to inactivate completely the action of catalase; the spores will still continue to contain some catalase, even when heated at 100°C. Treatment of a bacterial culture with chloroform and acetone may even stimulate its catalytic action but prolonged treatment will weaken it. In general, the resistance of bacterial catalase to disinfectants is distinct from that of the bacterial cells themselves (32).

The physiological rôle of catalase in the growth of an organism or a cell has been made the subject of numerous investigations. It is sufficient to mention the suggestion that oxidation processes in the organism or in the cell result in the formation of  $\text{H}_2\text{O}_2$ , which would become injurious were not the peroxide immediately destroyed by the catalase formed by the cells. Anaerobic organisms possess only a slight catalytic action and are rapidly injured by the addition of a small amount of hydrogen peroxide (9). Rywosch (28) found that although low concentrations of  $\text{H}_2\text{O}_2$  do not injure aerobic strains of *B. coli*, they are sufficient to kill rapidly the anaerobic strains of this organism. According to McLeod and Gordon (19), the inability of anaerobes to produce catalase, limits their growth in the absence of air. Kluyver (11) formulated the theory that those organisms which obtain their energy by the use of elementary oxygen contain catalase, whereas those that utilize the energy liberated by oxidation-reduction (fermentation) processes do not form catalase. Callow (6) tested nine anaerobic bacteria and twelve aerobic species; none of the anaerobes were found capable of producing catalase, whereas every aerobic form, except the streptococci, produced catalase. Stapp (32) found that both bacteria and yeast cultivated under anaerobic conditions were poorer in catalase than when grown in the presence of free oxygen. Virtanen and Karstrow (36), however, found that facultative anaerobic bacteria are richer in catalase when grown anaerobically, perhaps because the catalase is thus not used up for the decomposition of  $\text{H}_2\text{O}_2$ , which

is formed under aerobic conditions. Attention will be called later to the bearing that this phenomenon may have upon the catalytic power of the soil.

It is important to recall here the observation made by Morgulis and Levine (23) that benzol and a number of its homologues and derivatives act upon hydrogen peroxide in a manner similar to catalase. The decomposition of the peroxide is very violent, the maximum of the reaction being immediately attained and the reaction itself coming rapidly to a standstill. The action of these substances is thus distinctly different from that of catalase and of inorganic catalysts, which obey the laws of enzymatic and other catalyzed reactions. Similar observations have been made by Wu (41) on the catalytic action of hematin and all iron-containing derivatives of hemoglobin.

The contributions to the subject of the catalytic action of soils are meagre and, with very few exceptions, rather inconclusive. König and associates (12) reported that the catalytic action of soils is due to the action both of catalase and of inorganic catalysts, as shown in the following summary:

*Oxygen given off from 20 cc. of 2 per cent  $H_2O_2$  solution by 5 gm. of air-dried soil*

SOIL TREATMENT	INCUBATION	SANDY SOIL	LOAM SOIL	CALCAREOUS SOIL	CLAY SOIL
	<i>minutes</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>	<i>cc.</i>
Untreated.....	15	2	13	38	24
	60	4	32	80	51
	120	6	43	103	67
Ignited.....	15	...	1	2	1
	60	0.5	7	12	4
	120	0.5	16	26.5	10
Autoclaved 3 hours at 3 atmospheres.	60	3.0	13.5	23	21
Autoclaved 3 hours at 7 atmospheres.	60	5.0	15.5	13	26
Treated with chloroform.....	15	...	5	12	12
	60	...	11	15	18
	120	0.5	14	17.5	20
Treated with HCN.....	15	...	..	2	..
	60	...	6	7	1
	120	...	12	13	6

Ignited and autoclaved soils still continue to decompose  $H_2O_2$  because of the presence of sesquioxides. König and his associates (13) found that manganese and iron oxides are most active, whereas calcium and aluminum oxides are less active, 5 gm. of  $CaO$  liberating 20 cc. of oxygen in 1 hour, and 2 gm. of  $Mn_2O_3$  or  $Fe_2O_3$  liberating 140 cc. in 5 minutes. Since, however, these oxides are present in the soil in inconsiderable amounts, their action is probably negligible, May and Gile (18) having found that a clay subsoil of Porto Rico containing 29.3 per cent of ferric and aluminum oxides was almost devoid of catalytic action.

There seems to be, however, a definite correlation between the content of organic matter in the soil and its catalytic action, as shown by the following comparison:

*Catalytic power of the soil in relation to its "humus" content*

	SANDY SOIL	LOAM SOIL	CALCAREOUS SOIL	CLAY SOIL
"Humus".....	100	203	453	198
Oxygen liberated in 2 hours.....	100	721	1,721	1,117

The considerably greater increase of the catalytic power over that of the "humus" content is due to the presence of iron and manganese and other oxides in the heavier and calcareous soils. Similar results were obtained by Balks (2). Osuga (25) demonstrated that ferric oxide, manganese oxide, and "humus" show marked catalytic action; he suggested that these substances may be the main constituents which impart to the soil its distinct action upon hydrogen peroxide; he believed that the influence of bacteria upon soil catalysis was rather small, thus confirming the previous observations of Kappen (10). Stoklasa and associates (33) reached conclusions similar to those of König; the catalytic power of a soil containing the same amount of iron and manganese compounds increases with an increase in the biochemical activities, an increase in the fineness of soil structure and in alkalinity. Smolik (30) also attempted to find a correlation between the physical properties of the soil and its catalytic action; his results are, however, far from conclusive.

May and Gile (18) ascribed the catalytic action of soils to the presence of microorganisms and to their activities, especially in the decomposition of organic matter. Soils in which these activities were greatest were most active catalytically. The action of inorganic soil colloids was found to be negligible in comparison with the action of the enzyme. May and Gile suggested that the time required to bring about the evolution of a certain volume of oxygen from a certain quantity and concentration of peroxide should be used as a measure of the catalytic action of the soil. The addition of manures and fertilizers, however, did not seem to affect the activity and content of catalase in the soil even after 3 months. Sullivan and Reid (34) reported that the catalytic action of surface soils is greater than that of subsoils and that of fertile soils greater than that of infertile soils.

Chouchack (7) suggested a novel method of determining by its catalytic action the need of certain fertilizing elements in the soil. If a soil is deficient in a certain element it will respond to the application of that element by an increase in bacterial activity; the latter will manifest itself by an increase in the catalytic power of the soil, which can be used as a "biological index" of the need of soil for the elements essential for the growth of higher plants. This index was defined as the difference between the amount of oxygen liberated from a given amount of  $H_2O_2$  solution under a definite set of conditions by 4 gm. of soil before and after boiling for 1 minute. A series of samples of the soil in question receive an application of mannite and of the various fertilizing elements; moisture is then added to bring them to an optimum and the soils are incubated at 28°C. for 24 hours. Four-gram samples of the soil are then used for the catalytic test, which lasts for 15 minutes. The results seem to point to a parallelism between the change in the catalase content and the response in crop yield, as a result of the application of the various fertilizers. Chouchack (7) pointed out the advantage of this method over field experiments for determining the fertilizer requirements of the soil.

#### EXPERIMENTAL

The action of catalase is usually determined by adding a definite amount of soil to a certain quantity of hydrogen peroxide solution, adjusted to neutrality. The mixture is incubated at a definite temperature for a definite length of time and either (a) the residual peroxide is determined by titrating with  $KMnO_4$ , or (b) the amount of oxygen gas evolved in the reaction is measured. In some cases the time required for the evolution of a definite amount of oxygen, under standard conditions, is used as a measure. Morgulis (21) and Northrup (24), however, have shown that the concentration of the enzyme rapidly decreases with the time of action, and that the rate of the reaction is independent of the initial concentration of the peroxide. In the case of soil catalase, the problem is much more complex than in the case of animal or plant catalase, because of

the disturbing influence of the action of organic and inorganic complexes, non-enzymatic in nature. It is, therefore, best to limit the test to a definite set of conditions. In the following experiments, 5-gm. portions of air-dry soil were added to 20 cc. of a 1.5 per cent solution of  $H_2O_2$ , previously neutralized and warmed to  $37^\circ C$ .; the mixture was shaken 10 times and placed in a thermostat for 20 minutes at  $37^\circ C$ . Readings were taken at the end of 10 and 20 minutes. The gas is collected in burettes over a 2 per cent NaOH solution, so as to absorb the  $CO_2$  liberated in the process.

TABLE 1  
*Influence of various buffering agents upon the catalytic action of the soil*

SOIL TYPE	NATURE OF DILUENT*	OXYGEN GIVEN OFF IN 10 MINUTES
		cc.
Unlimed, acid soil.....	Water	15.1
	Phosphate mixture	17.0
Same soil, limed.....	Water	22.6
	Phosphate mixture	17.9
	Citrate mixture	24.3
	Acetate mixture	24.4
	Clark's solution	18.6

\* Reaction of acid soil, pH 5.2; of limed soil, pH 6.5. Sufficient buffer solution added to change the reaction of the soil to about pH 6.8-7.0.

TABLE 2  
*Influence of heat upon the catalytic action of the soil*

SOIL HEATED	OXYGEN EVOLVED AFTER	
	10 minutes	20 minutes
	cc.	cc.
Control.....	15.5	22.8
$50^\circ C$ ., 1 minute.....	8.3	14.4
$75^\circ C$ ., 1 minute.....	7.2	12.2
$100^\circ C$ ., 1 minute.....	5.0	8.1
15 pounds pressure, 15 minutes.....	0.5	0.7

Since it has been found that the reaction of the soil greatly influences the action of catalase, it was thought advisable to test first the effect of buffers upon the catalytic action of the soil. The results are given in table 1.

The use of the phosphate mixture at pH 7.0, with an acid soil of pH 4.8, resulted in a slight increase in its catalytic action, but the addition of phosphates to the limed soil resulted in an injurious effect upon the catalytic action. The injurious effect of the phosphate upon the catalytic action of the acid soil is more than balanced by the favorable effect exerted by making the soil

less acid. The use of acetates and citrates as buffers gave more favorable results.

To determine the influence of heat in the destruction of the catalase, so as to differentiate between the catalytic power of the soil due to the presence of the enzyme catalase and the action due to the inorganic substances of the soil capable of decomposing hydrogen peroxide, 5-gm. portions of soil were treated with 10-cc. portions of water and heated at different temperatures; 10-cc. portions of 3 per cent  $\text{H}_2\text{O}_2$  solution were then added and the oxygen liberated was determined. (Table 2.) Heating the moist soil for 1 minute was not sufficient to destroy all the catalase in the soil. It is actually necessary to autoclave the soil before the action of the catalase is destroyed.

TABLE 3  
*Influence of reaction upon the catalytic power of the soil*

TREATMENT OF 50 GM. OF SOIL	AFTER 2 DAYS						AFTER 15 DAYS		
	pH	Oxygen given off				pH	Oxygen given off		
		Before autoclaving		After autoclaving			10 minutes	20 minutes	
		10 minutes	20 minutes	10 minutes	20 minutes				
		cc.	cc.	cc.	cc.		cc.	cc.	
0.5N H <sub>2</sub> SO <sub>4</sub> , 10 cc.....	3.3	5.2	9.2	4.6	7.7	3.7	3.2	3.7	
0.5N H <sub>2</sub> SO <sub>4</sub> , 5 cc.....	3.4	9.5	14.0	2.1	3.6	4.1	4.7	6.6	
0.5N H <sub>2</sub> SO <sub>4</sub> , 2.5 cc.....	3.8	11.8	16.9	....	....	4.4	6.8	9.0	
0.5N H <sub>2</sub> SO <sub>4</sub> , 1.0 cc.....	4.4	16.9	26.7	...	...	4.8	8.6	9.3	
None.....	5.0	20.0	32.3	1.1	2.7	5.0	15.9	19.0	
CaO, 10 mgm.....	5.2	24.4	37.2	3.1	6.1	5.0	24.0	28.7	
CaO, 20 mgm.....	5.3	27.2	40.9	3.2	6.2	5.1	16.6	21.1	
CaO, 50 mgm.....	5.5	31.3	43.6	7.3	13.4	5.2	18.2	30.9	
CaO, 100 mgm.....	5.8	32.8	45.7	7.5	14.6	...	....	....	
CaO, 200 mgm.....	6.5	47.1	>50.0	12.5	19.0	...	....	....	
CaO, 500 mgm.....	8.2	>50.0		19.6	29.2	...	....	....	

Since the catalytic action of the soil was found to depend not only upon the presence of catalase and of certain inorganic soil constituents, but also upon the soil reaction, it was essential to find out how a change in the soil reaction influences the catalytic action of the soil as a whole and that of the enzyme catalase present in the soil. For this purpose, varying quantities of sulfuric acid and of CaO were added to 50-gm. portions of soil and the mixture was allowed to stand for 2 and 15 days, when the catalytic action was determined. (Table 3.) These results definitely indicate that increasing acidity rapidly depresses the action of catalase, whereas decreasing acidity stimulates the action of the enzyme. CaO itself acts catalytically, as seen by the gradual increase in catalytic action of the autoclaved soil. Subtracting the values obtained for the autoclaved

soil from those of the unautoclaved soil, however, shows that the "biological index" constantly increases with an increase in the pH value of the soil. Prolonged standing of the soil leads to a diminution in the catalytic action; this may be due to the fact that air-dried soil has been used for this experiment. Moistening of this soil brought an increase in the biological activities, whereas continued incubation of the moist soil led to a reduction of these activities.

Further information on the influence of hydrogen- and hydroxyl-ion concentration upon the catalytic action of the soil was obtained by heating black alkali soil (pH 9.6) with different portions of  $H_2SO_4$ , in order to adjust the reaction to different pH values; the catalytic action of the various soil portions was determined after 2 and 15 days. (Table 4.) A large part of the catalytic action of the untreated soil is thus found to be due to the inorganic soil constituents, namely the cations, as shown by the small decrease in action, as a result of autoclaving the soil. An increase in the hydrogen-ion concentration

TABLE 4  
*Influence of reaction of a "black alkali soil" upon its catalytic action*

FINAL REACTION	OXYGEN GIVEN OFF					
	After 2 days		After 15 days		After autoclaving	
	10 minutes	20 minutes	10 minutes	20 minutes	10 minutes	20 minutes
pH	cc.	cc.	cc.	cc.	cc.	cc.
9.6*	31.5	43.3	....	....	20.3	32.7
8.0	....	....	20.8	32.4	10.2	19.6
7.2	16.5	21.1	6.7	11.0	7.4	14.5
6.2	4.9	6.2	2.5	5.4	1.0	1.8
3.6	0	0	0.3	1.2	0.5	0.6

\* Control.

leads to a depression of the action of these inorganic catalysts. The same result can be accomplished not only by acids but also by appropriate buffering agents.

In order to determine how catalase may be actually formed in the soil, different substances were added under various conditions, so as to bring about an increased development of various groups of organisms in the soil. In the first experiment, 10-gm. portions of cellulose in the form of ground filter paper were added to a series of 1-kgm. portions of soil placed in pots; various amounts of water were then added so as to bring the moisture content of the soil to 30 per cent of its moisture-holding capacity, to 60 per cent, and to full saturation (about 35 per cent of soil weight). After 1 month incubation, the soils were tested for their catalytic action. (Table 5.)

The addition of cellulose to the soil was found to bring about only a slight increase in the catalytic power of the soil. An increase in moisture content led



to a decrease of the ability of the soil to liberate oxygen from  $\text{H}_2\text{O}_2$ , and ammonium sulfate also had a somewhat depressing effect, especially with a low moisture content. This experiment was repeated with practically identical results

TABLE 5  
*Influence of cellulose decomposition upon the catalytic action of the soil at different moisture contents*

CELLULOSE USED	NITROGEN SOURCE	MOISTURE	OXYGEN GIVEN OFF IN	
			10 minutes	20 minutes
			cc.	cc.
None . . . . .	None	10 per cent	26.8	41.9
		20 per cent	20.9	33.7
		Saturated	14.8	25.7
	Ammonium sulfate	10 per cent	20.0	30.8
		20 per cent	16.2	26.5
		Saturated	13.5	22.1
1 per cent . . . . .	None	10 per cent	29.5	42.9
		20 per cent	22.9	35.2
		Saturated	17.4	26.9
	Ammonium sulfate	10 per cent	29.2	39.0
		20 per cent	15.7	26.9
		Saturated	19.3	29.4

TABLE 6  
*Influence of cellulose and inorganic nitrogen compounds upon the catalytic action of the soil\**

CELLULOSE USED	NITROGEN SOURCE	OXYGEN GIVEN OFF IN	
		10 minutes	20 minutes
		cc.	cc.
None . . . . .	None	17.0	22.3
	Ammonium sulfate	16.5	22.0
	Sodium nitrate	16.3	21.5
1 per cent . . . . .	None	18.4	24.2
	Ammonium sulfate	17.9	24.4
	Sodium nitrate	20.0	26.3

\* Moisture content—25 per cent.

as far as cellulose is concerned. There was a less marked injury as a result of an addition of nitrogen salts, perhaps because of the relatively high moisture content. (Table 6.) The low catalytic power resulting from the addition of celluloses may be due to the fact that fungi are largely concerned in the decom-

position of celluloses in the soil (40) and to the fact, as shown by Dox (8), that many fungi produce only a very small amount of catalase.

It was pointed out above that Chouchack (7) found that the addition of

TABLE 7  
*Influence of mannite, buffer mixture and mineral elements upon the catalytic action of a soil*

SOIL TYPE	TREATMENT OF SOIL	PERIOD OF INCUBATION					
		2 days		18 days		49 days	
		Oxygen given off in		Oxygen given off in		Oxygen given off in	
		10 minutes	20 minutes	10 minutes	20 minutes	10 minutes	20 minutes
Acid soil	1 Dry soil	cc.	cc.	cc.	cc.	cc.	cc.
	2 Moist soil	11.6	17.4	....	....	....	....
	3 Soil + mannite	12.0	18.3	14.1	19.2	....	....
	4 Soil + mannite + 0.1 per cent $(\text{NH}_4)_2\text{SO}_4$	23.9	28.1	9.9	13.8	7.3	9.5
	5 Soil + 0.1 per cent $(\text{NH}_4)_2\text{SO}_4$	15.3	18.9	15.3	19.3	12.0	15.4
	6 Soil + mannite + 0.1 per cent $\text{Ca}(\text{H}_2\text{PO}_4)_2$	16.5	24.0	9.2	13.0	7.0	11.0
	7 Soil + mannite + 0.1 per cent $\text{K}_2\text{SO}_4$	19.1	22.8	13.0	17.1	8.0	12.0
	8 Soil + 0.5 per cent $\text{CaCO}_3$	15.8	18.1	....	17.8	10.2	13.5
	9 Soil + 2 cc. phosphate mixture	....	48.6	19.7	26.8	....	18.2
	10 Soil + 2 cc. sodium citrate-citric acid mixture	15.0	19.7	17.0	21.3	17.0	20.0
Limed soil	1 Dry soil	38.8	43.5	43.0	45.5	21.0	26.2
	2 Moist soil	11.7	18.3	....	....	10.5	16.7
	3 Soil + mannite	12.8	18.9	14.3	19.4	....	....
	4 Soil + mannite + 0.1 per cent $(\text{NH}_4)_2\text{SO}_4$	36.3	40.6	24.8	30.5	20.7	25.0
	5 Soil + 0.1 per cent $(\text{NH}_4)_2\text{SO}_4$	57.7	59.3	29.3	33.2	24.4	28.8
	6 Soil + mannite + 0.1 per cent $\text{Ca}(\text{H}_2\text{PO}_4)_2$	18.8	28.0	12.7	17.7	12.7	16.8
	7 Soil + mannite + 0.1 per cent $\text{K}_2\text{SO}_4$	34.5	38.2	24.5	28.5	17.5	21.6
	8 Soil + 0.5 per cent $\text{CaCO}_3$	57.3	60.0	33.5	37.7	23.6	28.0
	9 Soil + 2 cc. phosphate mixture	20.2	28.0	15.9	21.3	14.1	18.9
	10 Soil + 2 cc. sodium citrate-citric acid mixture	9.9	14.1	15.0	18.4	10.8	13.1
		55.7	60.5	28.6	36.5	18.7	23.8

mannite to the soil greatly stimulates its catalytic power, especially in the presence of minerals in which the soil is lacking. An experiment was, therefore, instituted to throw some light upon the influence of the addition of mannite upon the catalytic action of the soil, using an acid soil (A, pH 4.8) and a limed

soil (B, pH 7.0); 1 per cent mannite and 0.1 per cent portions of different minerals were added to a series of 100-gm. portions of soil. The optimum amount of moisture was then added, the soils were placed in covered tumblers and incubated at different intervals; 5-gram portions of soil were taken out and tested for the catalytic action. (Table 7.)

The results indicate very definitely that the addition of mannite brings about a decided increase in the catalytic action of the soil. This action is further increased, in the case of the limed soil, by the addition of nitrogen and mineral elements. On the other hand, the addition of  $\text{CaCO}_3$  increases the catalytic action of the acid soil, but only slightly that of the limed soil. It is interesting

TABLE 8  
*The catalytic action of cranberry peat soils*

NATURE OF SOIL	pH VALUE	OXYGEN GIVEN OFF BY 5 GM. OF SOIL			
		Untreated soil		Autoclaved soil	
		10 minutes	20 minutes	10 minutes	20 minutes
		cc.	cc.	cc.	cc.
Unlimed bog.....	4.0	38.5	47.1	1.5	3.2
3000 pounds of $\text{CaCO}_3$ per acre annually for last 3 years.....	7.1	25.2	32.2	1.6	3.2
Old bog, turfed.....	3.7	35.1	44.6	12.4	30.2
Old bog, unturfed.....	3.7	41.0	54.7	3.4	16.0
New bog, dark colored.....	3.8	45.3	57.3	15.8	19.3
New bog, brown.....	3.7	42.1	55.0	6.0	12.5
Plot with water table 18 inches below surface, 1-3 inches depth.....	3.6	53.2	83.9	2.3	3.9
Plot with water table 18 inches below surface, 10-12 inch depth.....	3.4	4.1	4.1	0.2	1.3
Plot with water table at surface.....	3.5	2.5	4.3	0.9	2.5
"Humus" from forest soil.....	...	39.4	41.3	....	....
0.5 gm. of mixed dry fungus mycelium.....	...	22.0	24.2	....	....

to compare the rapidity of the decomposition of the hydrogen peroxide by the soil to which mannite has been added with the action of the fungus mycelium and the acid peat and forest soils (table 8). This action is similar to that of the organic ring compounds investigated by Morgulis and Levine (23) and of the compounds studied by Wu (40). Perhaps we are not dealing here with an actual increase of catalase in the soil, but merely with the formation of certain organic compounds, either directly from the mannite or from the microbial protoplasm. The greater increase of the catalytic reaction in the limed than in the acid soil, as a result of an addition of mannite, is probably due to the fact that many bacteria (*Azotobacter*, etc.) would be capable of developing in the former and not in the latter soil.

The addition of phosphate mixture brought about a slight increase in the catalytic action of the acid soil but had a depressing effect on the limed soil. The addition of the citrate mixture increased the catalytic action of the acid soil even more markedly than that of the limed soil, perhaps partly because the citrate was used as a source of energy by the microorganisms.

Several cranberry peat soils from South Jersey were used (table 8) as a source of further information on the catalytic action of soils rich in organic compounds. To give 1 gm. of dry material, 5 to 20 gm. of soil was required, indicating the large amount of water that these soils can hold. The interesting result is that with a high water table, the catalase is absent, which may be accounted for by the fact that anaerobic organisms do not produce any

TABLE 9  
*Influence of soil treatment upon crop yield and catalytic power of the soil*

PLOT NUMBER	SOIL TREATMENT	TESTED JULY 20			TESTED SEPTEMBER 14			YIELD OF WHEAT PER ACRE, IN 1925	
		Oxygen given off in		Order	Oxygen given off in		Order	Pounds	Order
		10 min- utes	20 min- utes		10 min- utes	20 min- utes			
		cc.	cc.		cc.	cc.			
5A	Manure + minerals	21.2	27.1	1	17.1	23.1	1	7,251	2
7A	Nothing	8.9	11.8	10	10.6	14.4	8	393	12
9A	Minerals + NaNO <sub>3</sub>	14.2	18.3	4	12.1	16.4	4	6,263	4
11A	Minerals + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3.1	4.1	12	5.5	7.6	12	451	11
17A	Minerals + straw	8.4	12.8	8	12.8	16.0	5	4,145	7
18A	Minerals + manure + NaNO <sub>3</sub>	16.6	20.5	3	15.0	17.4	3	8,238	1
19A	Minerals only	7.7	10.1	11	10.6	12.5	11	3,141	9
4B	Minerals + lime	11.2	14.5	7	10.6	14.0	9	3,175	8
5B	Minerals + manure + lime	17.3	21.5	2	14.4	18.8	2	4,973	5
7B	Lime only	13.9	17.1	5	10.8	15.7	6	2,846	10
11B	Minerals + (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> + lime	7.8	12.2	9	9.7	13.2	10	6,503	3
19B	Minerals only	11.8	16.1	6	11.3	15.3	7	4,669	6

catalase or that the enzyme is destroyed when the soil is saturated with water.

It is rather difficult to explain these results, unless the catalase content of the plants and the nature of the organisms contributing to the decomposition of these plants are known. It is interesting to note the explosive character of the "humus" forest soil and of the fungus mycelium.

The nature of the catalytic action of the soil is very complex because of (a) the inorganic constituents of the soil, which seem to be especially active in alkaline soils; (b) the organic constituents, especially abundant in forest soils and in other soils rich in organic matter; and (c) the enzyme catalase, the amount of which depends on the nature of the plant residues and on the

microorganisms active in the decomposition of these plant residues; as well as on (d) the reaction and moisture content of the soil. The results obtained by the application of such a method would, therefore, be too complex to be used as a basis of comparison among different soils. An attempt was made, however, to use this index for determining the biological condition of a series of soils from various plots under a definite system of fertilizer treatment for the last 18 years, as reported by Lipman and Blair (26) and in the previous papers in this series (37-39). The results are given in table 9.

The limed plots show a considerably higher catalytic power than the unlimed plots. This would be expected from the previous observations on the influence of reaction upon the catalytic action of the soil. These results are also comparable with those reported elsewhere on the numbers of microorganisms and their activities in the soil. Plot 5B is an exception, since it has a high catalytic power and gave a comparatively low crop yield; however, when the crop yields of this plot for the previous years are considered, it is found to be one of the highest yielding plots. The yields are recorded as dry matter, including both straw and grain. Plot 5B gave a considerably greater proportion of grain to straw than plot 18A, for example. Plot 11B, receiving ammonium sulfate and lime, gave a very high yield but a low catalytic power, because of its comparatively acid reaction, which acts injuriously upon the catalase. With these two exceptions, which can be readily explained, the parallelism between the catalytic power and the crop yields is rather interesting.

#### SUMMARY

1. The liberation of oxygen from hydrogen peroxide by soil is a result of three distinct processes:

- (a) The action of the enzyme catalase, which may be either of plant or microbial origin, the latter usually predominating.
- (b) The catalytic action of certain organic substances which seem to be found in great abundance in dead fungus mycelium and in soil rich in organic matter.
- (c) The action of inorganic catalysts, especially in alkaline soils.

2. An acid reaction is unfavorable to the catalytic action of the soil, whereas a neutral or slightly alkaline reaction is favorable. One must differentiate of course between the influence of the hydrogen-ion concentration of the soil upon the actual amount of catalytic agent formed or present in the soil and upon the course of action of this agent. A high moisture content is injurious to the catalytic action of the soil.

3. There is a certain parallelism between the crop-producing capacity of a soil and its catalytic action. It is doubtful, however, whether this method can be considered as giving information upon microbiological processes in the soil, since frequently the catalytic action is due not so much to the microorganisms of the soil, as to certain inorganic or organic constituents which may be present in abundance in certain soils.

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# ON THE ORIGIN AND NATURE OF THE SOIL ORGANIC MATTER OR SOIL "HUMUS": V. THE ROLE OF MICROÖRGANISMS IN THE FORMATION OF "HUMUS" IN THE SOIL<sup>1</sup>

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The previous investigations seem to point to the fact that when organic matter is added to the soil, the soluble carbohydrates, proteins, pentosans and other hemicelluloses, as well as the true celluloses are readily decomposed, leaving the lignins (and probably also some of the fats and waxes) to accumulate in the soil. The assumption that lignin is the only mother substance of the soil "humus" is hardly sufficient, however, to account for the nature of this "humus," especially since the latter is usually found to contain 2.0 to 3.5 per cent nitrogen, whereas pure lignin is free from nitrogen. The preparations of lignin, obtained both by treatment with the concentrated acid and by extraction with sodium hydroxide, however, seem to contain a small amount of nitrogen. The nitrogen part of the "humus" could originate either from the proteins of the organic matter added to the soil or through the synthesizing activities of microörganisms. The former could hardly account for such a large content of nitrogen: first, because most of the natural organic substances contain only a small amount of nitrogen (18 to 20 per cent of lignin and only 0.4 to 0.5 per cent of nitrogen in straw), and secondly, the addition of proteins and of amino acids to the soil greatly stimulates the activities of various groups of microörganisms and results in the rapid decomposition of the proteins with the formation of ammonia. Lathrop (2) and others called attention to the fact that when proteins are added to the soil, they are decomposed but there is, at the same time, a formation of a new protein material which is more resistant to decomposition. This protein material could only be of microbial origin. The idea of Maillard and others (3) that "humus" originates from the chemical interaction of sugars and amino acids can hardly explain this process, since there is at no time in normal soil more than mere traces either of sugars or of amino acids, both of which are rapidly used up by different groups of micro-organisms.

It has been pointed out previously that the decomposition of non-nitrogenous substances by microörganisms in the soil leads to an extensive synthesis of new microbial protoplasm, as indicated by the abundant assimilation of inor-

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ganic nitrogen and its transformation into microbial proteins and other complex nitrogenous substances. If fungi are the active agents of decomposition, 1 part of nitrogen will be assimilated for every 7 to 8 parts of carbon given off as  $\text{CO}_2$ , in the process of energy utilization. If pure cultures of bacteria are the active agents in the decomposition of celluloses and other non-nitrogenous substances, 1 part of nitrogen will be assimilated for 12.5 parts of carbon liberated as  $\text{CO}_2$ . Since bacterial cells are richer in nitrogen than fungus mycelium, a much smaller amount of protoplasm will be synthesized. If the mixed soil flora and fauna take part in the active processes of decomposition of the organic matter added to the soil, 1 part of nitrogen will be transformed from an inorganic into an organic form for every 16 to 18 parts of carbon given off as  $\text{CO}_2$ .

The last process should be the one to be emphasized most, since it considers the soil flora and fauna as a whole, unmodified. It may be assumed that, in the process of decomposition of plant stubble and straw, 60 to 80 per cent of the carbon is decomposed by the soil organisms, in a relatively short period of time, leaving 20 to 40 per cent of the carbon, which comprises the lignins and some of the fats and waxes, to persist in the soil for a longer period of time. As a result of decomposition of the 60 to 80 per cent of the carbon, about 40 to 70 per cent is given off as  $\text{CO}_2$  and 10 to 20 per cent left in the soil in the form of microbial protoplasm. This can be readily checked up by another process of reasoning. If, out of 100 units of carbon added to the soil in the form of natural plant residues, 40 to 70 units are given off as  $\text{CO}_2$  and if 1 unit of nitrogen is assimilated by the organisms for every 16 to 18 units of carbon given as  $\text{CO}_2$ , 2.5 to 4 units of nitrogen will be assimilated. Since the microbial protoplasm contains 5 to 10 times as much carbon as nitrogen, the assimilation of 2.5 to 4 units of nitrogen will necessitate the assimilation of 10 to 40 units of carbon—probably on the average, 10 to 20 units—since the mycelium of the fungi and the cells of the bacteria, which are the agents assimilating considerable quantities of carbon, would soon be partly decomposed by the other bacteria and actinomyces, liberating a part of the carbon of the mycelium as  $\text{CO}_2$ . This  $\text{CO}_2$  is included already in the total amount considered above for the complex soil population.

These calculations show that very considerable quantities of organic matter—as much as 10 to 20 per cent of the total amount introduced into the soil—are synthesized in the soil by microorganisms. This synthesized organic matter is very rich in nitrogen, containing as much as 5 to 10 per cent, and is no doubt sooner or later decomposed by the various representatives of the soil flora and fauna. A part is changed into  $\text{CO}_2$ ,  $\text{NH}_3$ , etc.; a part is utilized by other organisms and changed into fresh microbial protoplasm; and a part, being resistant to decomposition, is left in the soil. This last part contributes to the soil organic matter or "humus" and is of great interest in connection with these studies on the origin of "humus" in the soil. A series of preliminary experiments were undertaken with this idea in mind.

## EXPERIMENTAL

*Experiment 1. The formation of "humus" in sand media*

The first experiment on the synthesis of "humus" was carried out using sand as a medium, so that the large quantities of organic matter commonly found in the soil should not interfere in the attempt to establish how much "humus" is actually synthesized. Eight-hundred-gram portions of washed white sand were placed in a series of small earthenware pots. These were divided into 3 groups: one group of pots received 2 per cent of rye straw, then, after 35 days, again 2 per cent of straw; the second group received also the two treatments of straw on the same respective dates and, in addition, 2 gm. of  $(\text{NH}_4)_2\text{HPO}_4$ , 0.5 gm.  $\text{MgSO}_4$ , and 0.5 gm.  $\text{KCl}$  per pot at each treatment; the third group of pots received two additions of 2 per cent ground filter paper and the same amount of inorganic salts as the second group. The moisture content of half of the cultures in each series was brought to optimum (about 20 per cent moisture), while the sand in the other half of the pots was saturated with water, allowing a layer of free water about 2 cm. deep to cover the surface of the sand. The pots were inoculated with a dilute suspension of a rich garden soil, covered with glass plates and incubated at 25 to 28°, water being added at frequent intervals to bring to original weight. The cultures were analyzed 60 days after the second addition of organic matter.

The contents of the pots were well mixed, and aliquot portions of the moist mixture (including portions of the excess liquid where an excess of water was added) taken out into a series of flasks or beakers. The residual organic matter in one portion of the sand mixture can be readily removed from the sand by constant washing upon a filter paper, the dry weight of which has previously been determined. At first normal  $\text{KCl}$  is used for washing, so as to remove all the residual ammonia; after about 250 cc. of the filtrate has been collected from about 100 gm. of sand culture, distilled water is used for further washing. Ammonia is determined in the filtrate by adding some  $\text{MgO}$  and distilling into standard acid. The sand is well stirred with consecutive portions of water until all traces of organic matter are removed. Some of the sand is of course also transferred thereby upon the filter. The paper, containing the organic matter with the adhering sand is dried at 80 to 90°C. to constant weight. The contents of the paper are then carefully transferred to an Erlenmeyer flask. An equal amount of 5 per cent  $\text{NaOH}$  solution is then added and the flasks are autoclaved, at 15 pounds pressure, for 30 minutes. The contents of the flask, while still hot, are diluted with an equal quantity of distilled water and transferred carefully upon weighed ashless filter papers. Every particle of sand in the flask is washed upon the filter; the residue is washed several times with water, dried to constant weight, at 80 to 90°C., and ignited. The ash subtracted from the weight of the original residue gives the amount of organic matter left in the sand, free from ash and water-soluble constituents; the weight of the ash subtracted from the second residue gives the amount of

organic matter not soluble in 5 per cent NaOH solution. The NaOH solution and the washings are treated with an excess of 1:1 HCl. The precipitate is filtered, washed, dried, weighed, and ashed. The ash-free precipitate is recorded as "humus" (this preparation is the  $\alpha$ -fraction of soil "humus," which is practically ash-free).

This method has one distinct disadvantage, namely, in the process of removing the organic matter from the sand by washing, all the water-soluble substances, including a large part of the bacterial cells, are washed out. By determining the total carbon and the total nitrogen content of the combined filtrate, however, the organic matter thus removed is measured. The "humus" was also extracted directly from aliquot portions of the sand culture (contain-

TABLE 1

*Formation of "humus" from straw and cellulose in sand media, under aerobic and anaerobic conditions\**

On the basis of 100 gm. of dry residue

TREATMENT			TOTAL NITROGEN	TOTAL AMMONIA NITROGEN	RESIDUAL ORGANIC MATTER FREE FROM ASH AND THE WATER-SOLUBLE PORTION	RESIDUAL CELLULOSE	ORGANIC MATTER EXTRACTED BY NaOH (5 PER CENT SOLUTION)	"HUMUS" $\alpha$ -FRACTION FREE FROM ASH	
Nature of organic matter (4 per cent used)	Moisture	Nitrogen and minerals						Total weight	Nitrogen content
	<i>per cent</i>		<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>per cent</i>
Straw.....	60	0	37.8	Trace	2,237	346	1,436	474	2.06
	100	0	36.8	Trace	2,918	1,065	1,603	636	1.84
	60	+	111.4	49.2	2,263	258	1,513	520	1.98
	100	+	110.6	53.2	2,705	376	1,647	621	1.70
Cellulose.....	60	+	92.0	34.8	1,720	609	921	106	4.95
	100	+	86.6	47.7	3,618	3,208	111	30	....

\* The ash-free, dry organic matter added in the case of straw was 3300 mgm. and in the case of cellulose 3700 mgm.

ing the organic matter) by treating with 5 per cent NaOH solution, then filtering and precipitating the "humus" ( $\alpha$ -fraction) as usual. Cellulose and total nitrogen were determined in aliquot portions. Separate portions were used for moisture determinations and the results were all calculated on the basis of dry weight of the sand culture.

The data recorded in table 1 give an interesting insight into what is taking place when organic matter is decomposed in the soil. Out of 3300 mgm. of organic matter—free from moisture, ash, and water-soluble constituents—added to 100 gm. of sand in the form of straw, about 2250 mgm. was left under aerobic conditions, and about 2800 mgm. under anaerobic conditions, showing a much more rapid decomposition in the presence of sufficient aeration. The greater decomposition of the organic matter under aerobic conditions is largely

accounted for by the amount of cellulose decomposed. It was pointed out elsewhere (4) that bacteria capable of decomposing cellulose under anaerobic conditions are lacking in normal soils. When cellulose is introduced into a normal soil and sufficient water is added to make conditions anaerobic, no cellulose decomposition will take place before 2 or 3 months have elapsed, because of either a gradual development of the proper organisms or a gradual adaptation of a proper population already existing in the soil. Once such a population has become established, however, the celluloses will be rapidly decomposed. The excess of cellulose (determined directly) in the sand culture kept under anaerobic conditions over the aerobic soils accounts quantitatively for the smaller amount of organic matter decomposed.

By comparing the figures for residual organic matter, for residual cellulose, and for the organic matter which is soluble in 5 per cent sodium hydroxide, one can readily see that a large part of material has been synthesized in the process of decomposition of organic matter added to the sand medium, especially under aerobic conditions. In the case of straw kept at an optimum moisture content, out of the 2250 mgm. of organic matter (free from ash and water-soluble constituents) left, about 1520 mgm. is soluble in sodium hydroxide solution, and only 730 mgm. is insoluble. The insoluble portion consists of about 300 mgm. of cellulose, as determined quantitatively, and 430 mgm. of organic matter unaccounted for, including probably some lignins, fats, waxes, and largely pentosans. The original straw (free from ash and water-soluble materials) contained only about 30 per cent of material extracted by sodium hydroxide. This has increased, as a result of decomposition processes, to 67.5 per cent. This increase is due both to the relative increase of the lignins, which have not been decomposed to any extent, and to the substances synthesized by the microorganisms, which have utilized the celluloses as sources of energy. A large part of this protoplasm is soluble in sodium hydroxide and is precipitated by hydrochloric acid. The "humus" formed from the decomposition of straw in sand is thus due both to the substances of plant origin and to the synthesizing action of microorganisms.

This synthesizing action is brought out especially in the data obtained from the decomposition of pure cellulose in the sand cultures. Originally the sand and paper did not contain any substances soluble in dilute alkalis. The rapid decomposition of the cellulose under aerobic conditions resulted in a reduction of the cellulose from 3700 mgm. (pure, water-free cellulose) to 609 mgm. Of the 3091 mgm. of cellulose that has become decomposed, there is formed in the soil 1011 (1720 — 609) mgm. of organic matter, over 90 per cent of which (921 mgm.) is soluble in sodium hydroxide. That this newly formed organic matter is synthesized microbial protoplasm can be confirmed by the nitrogen data; the pots, in which cellulose has decomposed under aerobic conditions, contain 92.0 mgm. of total nitrogen (in 100 gm. of dry sand medium) and 34.8 mgm. of ammonia nitrogen, or 57 mgm. of nitrogen in the form of synthesized organic matter. This amounts to about 5.6 per cent nitrogen for

the synthesized organic matter in the soil. It is important to remember, in this connection, that fungus mycelium contains about 4 to 8 per cent nitrogen, with an average of about 5 to 6 per cent, as shown by Heukelekian and Waksman (1) in the study of decomposition of cellulose by pure cultures of fungi. In other words, *for 3091 mgm. of nitrogen-free cellulose decomposed, there has been synthesized 1011 mgm., or about one-third, of microbial protoplasm which contains 5.6 per cent nitrogen, and 90 per cent of which is soluble in sodium hydroxide.* These results show definitely that sodium hydroxide extracts from normal soils a complex mixture of organic materials or "humus" which consists largely of a nitrogen-poor lignin, introduced with the original organic matter added to the soil and which is resistant to decomposition, and a nitrogen rich constituent of microbial protoplasm synthesized in the soil.

This "humus" formation is illustrated in the data on the  $\alpha$ -fraction of the "humus." It must be kept in mind that this is the part of the organic matter which is dissolved by the sodium hydroxide and precipitated by an excess of hydrochloric acid, in other words, that part of the soil organic matter which has most commonly been referred to as "humic acid." When washed with sufficient dilute hydrochloric acid and water, this fraction is practically free from ash. It has been pointed out elsewhere that, when obtained from normal cultivated soil, the nitrogen content of this  $\alpha$ -fraction or "humic acid" is about 2.5 to 3.5 per cent. The amount of "humic acid" obtained from the sand medium, in which straw has been decomposing under aerobic conditions, is somewhat less than under anaerobic conditions, due to the fact that, with sufficient aeration, certain bacteria and actinomyces are capable of breaking down the lignin very slowly. Under anaerobic conditions, these organisms are not very active and the lignin passes largely into soil "humus." The nitrogen part of the "humus" is largely a result of the synthesizing activities of the microorganisms of the soil, as shown by the data on the "humus" formed from cellulose: the decomposition of about 3 gm. of cellulose, in the form of filter paper, resulted in the synthesis of about 1 gm. of organic matter, derived from the microbial cells, over 10 per cent of which gives all the characteristics of "humic acid," or the  $\alpha$ -fraction of "humus." This synthesized "humic acid" contained 4.7 per cent nitrogen. The "humic acid" found in the pots receiving straw has been derived largely from the lignin of the straw and the synthesized microbial cells; the latter have contributed the nitrogen-bearing fraction; the total nitrogen content in the "humus" is considerably less under anaerobic conditions where less protoplasm has been synthesized. This is because, under aerobic conditions, fungi and aerobic bacteria are largely responsible for the decomposition of celluloses and under anaerobic conditions bacteria carry on this process; since the former synthesize more protoplasm than the latter, the "humic acid" or the  $\alpha$ -fraction of "humus" found under aerobic conditions will contain more nitrogen, especially before that "humus" begins to decompose, in its turn.

Under anaerobic conditions, where fungi and aerobic bacteria are less active or are inactive, decomposition of the added organic matter proceeded much

more slowly, especially in the case of the celluloses. Although the total amount of organic matter decomposed under anaerobic conditions is considerably less than under aerobic conditions, more of the "humus," or of that part of alkali extractable material which is precipitated with an excess of hydrochloric acid, is left under anaerobic conditions. This is due largely to the fact that although the lignin may be somewhat decomposed under aerobic conditions, by the action of certain organisms—this question will be discussed in detail later—very little of it is decomposed under anaerobic conditions. A considerably smaller amount of "humus" is synthesized in the last case, as shown by the data for the decomposition of the ground filter paper.

*Experiment 2. The formation of "humus" in the soil*

The formation of "humus" in soil itself was studied in a manner similar to the studies in sand media, but the studies were continued for a considerable time. A rich sandy sassafrass soil was air dried, and 1000-gm. portions were placed in a series of glazed earthenware pots. Since the soil was acid, 2 gm. of  $\text{CaCO}_3$  was added to each soil, thereby bringing the reaction to pH 6.2. A few of the pots with soil were left without organic matter, a few received an application of 1 per cent cellulose (in the form of filter paper) and 0.1 per cent  $\text{NaNO}_3$ , and a few received 1 per cent straw and 0.1 per cent  $\text{NaNO}_3$ . The soil moisture in half the pots was brought to 60 per cent saturation and in the other half to full saturation, allowing an excess of water over the surface so as to make conditions anaerobic. Water was added at weekly intervals to keep the moisture at the same concentration. The pots were covered with glass plates and incubated at 25 to 28°C. After 5 months incubation, when most of the cellulose had been decomposed, 2 per cent filter paper and 0.2 per cent  $\text{NaNO}_3$  were added to the cellulose pots and 2 per cent straw to the corresponding soils. The soils were again incubated for 10 weeks, and then analyzed. The cellulose determinations have shown that only about 0.1 per cent cellulose was left in the aerobic soils and 1.16 per cent in the anaerobic soils because of the abundant activities of the fungi in the former soil.

The  $\alpha$ -fraction of the "humus" is that part of the soil organic matter which is soluble in alkalis (5 per cent  $\text{NaOH}$ ) and is precipitated by an excess of acid. The  $\beta$ -fraction of the "humus" is that portion which is soluble both in an alkaline and in an acid solution and which is precipitated at a pH value of about 4.8, as shown before. (Table 2.)

The results seem to confirm quite definitely the observations made upon the formation of "humus" in the sand media. The soil itself contains a considerable amount of organic matter. It is interesting to note, in this connection, that the soil kept under aerobic conditions for 7½ months had less organic matter in the "humus" fractions than the soils kept under anaerobic conditions. This is due entirely to the fact that "humus" is not decomposed to any extent when the soil is saturated with water, because the organisms capable of bring-

ing about this process are aerobic and are favored by a lower moisture content and by a neutral reaction—facts to be dwelt upon in a later contribution. This serves to throw interesting light upon the accumulation of organic matter (“humus”) in soils kept saturated with water—as peat soils—as well as upon the rapid decomposition of organic matter in arid soils. The decomposition of the “humus” under aerobic conditions can be readily measured also by the formation of carbon dioxide and by the accumulation of nitrate nitrogen. Both of these phenomena run parallel because of the nearly constant ratio between the carbon and nitrogen in the soil. Since these soils were kept in closed containers, the nitrates accumulated and the excess of nitrate and ammonia nitrogen in the aerobic soil over the anaerobic soil more than balanced the lower nitrogen content in the two “humus” fractions in the aerobic soils. Under natural conditions, this soluble nitrogen would either be assimilated by

TABLE 2

*Formation of “humus” in the soil from cellulose and straw, under aerobic and anaerobic conditions*  
On the basis of 100 gm. of dry soil

NATURE OF ORGANIC MATTER (3 PER CENT)	MOISTURE	“HUMUS,” $\alpha$ -FRACTION, ASH FREE		“HUMUS,” $\beta$ -FRACTION, ASH FREE		TOTAL “HUMUS”	TOTAL NITROGEN IN “HUMUS”
		Total in 100 gm. of soil	Nitrogen content	Total in 100 gm. of soil	Nitrogen content		
	<i>per cent</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>
Control.....	60	533	13.0	1,110	10.0	1,643	23.0
	100+	470	12.5	1,555	16.5	2,025	29.0
Cellulose.....	60	668	18.5	1,253	11.0	1,921	29.5
	100+	575	18.3	1,518	17.8	2,093	36.1
Straw.....	60	920	18.3	1,635	14.6	2,555	32.9
	100+	1,028	19.3	1,818	23.0	2,846	42.3

higher plants—wild or cultivated—or would be leached out. One of the reasons for the accumulation of “humus” under anaerobic conditions is the insufficient decomposition of the substances of plant (lignins) and microbiological origin.

The soils that have received an application of 3 per cent cellulose and sufficient nitrate nitrogen for the complete decomposition of the former, show a gain in “humus,” especially under aerobic conditions. Cellulose is completely decomposed by microorganisms (1) under aerobic conditions, leaving only synthesized microbial protoplasm and one waste product—carbon dioxide. It is this synthesized protoplasm which contributes to the soil “humus,” as demonstrated in the previous experiment and brought out also in table 2. The fact that the gain in the “humus,” especially in the  $\alpha$ -fraction, is directly of fungus origin can be illustrated by the increase in the nitrogen content in

this fraction—a gain of 135 mgm. in the  $\alpha$ -fraction of 100 gm. of soil was accompanied by a gain of 5.5 mgm. of nitrogen in that fraction, or a little more than 4 per cent nitrogen. Where the gain in "humus" is due only to an increase in lignin content, there is a tendency to a diminution of the nitrogen content, since lignin is free from nitrogen; where the gain in "humus" is due both to lignin and to constituents of the microbial protoplasm, as when natural plant materials are added to the soil, there is a balance in the nitrogen content of the "humus," especially of the  $\alpha$ -fraction. Under anaerobic conditions, cellulose is decomposed entirely by bacteria which synthesize only a very small amount of protoplasm under these conditions. The total gain in the amount of "humus" is very small, although an appreciable gain is found in the  $\alpha$ -fraction, accompanied by a gain in the nitrogen content of this fraction.

The greatest gain in "humus" is found in the soils receiving 3 per cent of straw, especially under anaerobic conditions. A number of phenomena speak here for the contribution of the lignin to the soil "humus":

1. The increase in the "humic acid" ( $\alpha$ -fraction) under anaerobic conditions is 558 mgm. (1028 — 470); this is equivalent quantitatively to the amount of lignin added, the straw containing 18.8 per cent lignin ( $188 \times 3 = 564$ ). It should be noted that the lignin comes down in the "humic acid" fraction and is not decomposed under anaerobic conditions; under aerobic conditions, there is a smaller increase in the "humic acid" because part of the lignin was no doubt decomposed.

2. Although the increase in the  $\alpha$ -fraction of the "humus" ("humic acid") in the soils receiving the straw is much greater than in the soils receiving the pure cellulose (nearly three times as much under aerobic and over five times as much under anaerobic conditions), there is practically no gain in the nitrogen content. This is due to the fact that lignin theoretically contains no nitrogen; the gain in the nitrogen content of the "humus" comes from the ingredients of the microbial protoplasm contributing to the "humus" and to some extent of the protein of the straw. Since straw contains only about 70 to 75 per cent of the energy available for the activities of the soil microorganisms (monosaccharides, starches, celluloses, pentosans) that is offered by an equivalent amount of filter paper, a proportional amount of microbial protoplasm can be synthesized under the same conditions.

Straw contributes to the soil "humus," in addition to the lignins which are found in the  $\alpha$ -fraction, a certain ingredient in the  $\beta$ -fraction, especially under aerobic conditions. Whether this is a constituent of the straw or a synthesized material which is absorbed by the aluminum of the  $\beta$ -fraction remains to be investigated.

The results of this experiment, as well as those of the previous one, point definitely to the fact that the  $\alpha$ -fraction of the humus is formed in the soil by the lignin from the higher plants (and to a more limited extent by the fats and waxes) and by certain ingredients of the synthesized cells of microorganisms. These ingredients contribute largely to the nitrogen part of this "humus" fraction. The cells have been synthesized as a result of the utilization of the celluloses, pentosans, starches, monosaccharides, and other constituents of plant tissues, as sources of energy by the microorganisms, and the proteins or of available nitrogen compounds as sources of nitrogen.



*Experiment 3. Fungus mycelium as a source of humus in the soil*

To learn more exactly the rôle of the cells of microorganisms as contributing agents to the soil "humus," the following experiment was carried out:

A quantity of mycelium of a green *Trichoderma*—a common cellulose-decomposing organism in the soil—and of *A. niger*—a common mold in nature—was prepared by growing the organisms upon a synthetic (Czapek's) solution in large flasks until growth was at a maximum. The mycelium was then filtered off through paper, washed with distilled water, and dried. Two quantities of mycelium were thus obtained. These were treated by the same procedure as was the straw described in the previous paper; namely, first they were extracted with ether, then with 95 per cent alcohol, then with cold water for 24 hours, and then with 5 per cent NaOH for 48 hours at room temperature,

TABLE 3  
*Composition of fungus mycelium\**

ORGANISM	TRICHODERMA	ASPERGILLUS
	<i>per cent of total</i>	<i>per cent of total</i>
Untreated mycelium.....	(I)	(II)
Moisture content.....	7.6	6.40
Ether extract.....	4.2	.....
Alcohol extract.....	8.1	8.00(III)
Cold water extract.....	17.3	15.10(IV)
5 per cent NaOH extract.....	29.8	30.32(VI)
Loss on extraction with H <sub>2</sub> SO <sub>4</sub> .....	17.8	16.18
Insoluble residue.....	15.2	24.00(VII)
NaOH extract precipitated with HCl.....	20.3	23.02(VIII)

\* The Roman numerals designate the number of the preparation, obtained as a result of the particular treatment (as VI = preparation of *Aspergillus* mycelium, after NaOH extraction).

and finally they were treated with 2 per cent H<sub>2</sub>SO<sub>4</sub> for 1 hour at boiling temperature.

Table 3 gives the analysis of the two fungus preparations. Ether was found to extract 4.2 per cent of the fungus mycelium, alcohol 8.0 to 8.1 per cent, and cold water 15.1 to 17.3 per cent. Five per cent sodium hydroxide extracted 29.8 per cent of the *Trichoderma* mycelium and 40.32 per cent of the *Aspergillus* mycelium, the larger part of which could again be precipitated by hydrochloric acid; in other words, the preparations of both fungi contained 20.3 and 23.02 per cent of material which comes down as the  $\alpha$ -fraction of "humus" or as "humic acid." The nitrogen contents of these fractions were 4.31 and 3.33 per cent respectively. It is interesting to compare these data with those reported by Winterstein and Reuter (5) for higher fungi. These investigators reported 4 per cent ether extractives (3.3 per cent fat and 0.5 per cent cholesterol), 12.0 per cent alcohol extractives (sugars, lecithin, bases, amino

bodies, purine bodies), and 28.0 per cent water-soluble substances (sugar, glycogen, purine bases, amino acids, etc.); the residue, comprising 46 per cent, was found to consist of 30 per cent protein, 10 per cent of an amorphous carbohydrate (para-iso-dextrin), and 6 per cent chitin.

Preparation VII, or the residue insoluble in dilute alkalis and dilute acids, probably consists largely of chitin. The presence of this preparation in the soil probably has something to do with the substance usually described as "humin" and "ulmin," or the most resistant part of the soil organic matter, as far as treatment with chemicals is concerned. It is comparable to cellulose, but it contains nitrogen and decomposes readily in the soil—to be sure more slowly than the soluble carbohydrates, starches and proteins, but it does not accumulate in the soil. It was not the purpose of this experiment to isolate any specific compounds from microbial protoplasm and to study their decomposition in the soil, but merely to separate the mycelium into several fractions with the idea of learning which of these decomposes more readily and whether any ingredients which may contribute to the soil "humus" are left in the soil.

Two-gram portions of the different preparations were added to a series of 100-gm. portions of washed sand or soil placed in long necked flasks. The sand cultures received also 20 mgm. of nitrogen in the form of dibasic ammonium phosphate and a trace of  $MgSO_4$  and KCl. The sand cultures were sterilized and inoculated with a pure culture of *Trichoderma*, while the soil cultures were left unsterilized. The cultures were placed in the incubator (at 27 to 28°C.) and connected with the respiration apparatus; the  $CO_2$  evolved was absorbed in standard barium hydroxide solution. After 34 days' incubation, the cultures were analyzed for residual ammonia, organic matter (in the sand cultures) and "humus" fraction in the soil.

The results indicate quite definitely that fungus mycelium, as represented by two of the most common filamentous fungi in the soil and in nature, consists of fractions which decompose with different degrees of rapidity. The removal of the ether-soluble fraction increases the degree of decomposition of the mycelium. The removal of the water-soluble fraction, however, tends to reduce the degree of decomposition of the mycelium; ether extracts substances which decompose with difficulty; while water removes the soluble sugars and amino acids which decompose readily. The most interesting portion is that removed by sodium hydroxide; it is largely nitrogenous in nature as indicated by the fact that whereas the mycelium of *Trichoderma* contained only 4.8 per cent nitrogen and the mycelium of *A. niger* 3.67 per cent nitrogen, the material extracted by sodium hydroxide contained 7.9 per cent nitrogen. The preparation obtained by precipitating this extract with hydrochloric acid contained 4.3 to 6.0 and 3.3 to 5.0 per cent nitrogen, respectively. The removal of this preparation does not prevent the decomposition of the residual material but reduces it somewhat in the sand culture and even increases it in the soil culture (fraction VI). The fraction left, after the alkali treatment, contains a considerable quantity of carbohydrate, amylose in nature, a large part of which

TABLE 4  
*The decomposition of fungus mycelium in sand by Trichoderma and in soil by mixed soil flora*

NUMBER OF PREPARATION	MEDIUM	CARBON EVOLVED AS CO <sub>2</sub> —INCUBATION						C LIBERATED AS CO <sub>2</sub> —CONTROL SUBTRACTED	AMMONIA N + NITRATE N CONTENT IN 100 GM. OF SAND OR SOIL	AMMONIA N FORMED FROM MYCELIUM	"FUMUS" ( $\alpha$ AND $\beta$ FRACTIONS) IN 100 GM. OF SOIL	
		2 days	5 days	10 days	15 days	21 days	34 days				Total	Control subtracted
		mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.	mgm.
I	Sand	24.5	44.4	45.1	73.7	36.6	7.9	234.2	47.2	27.2		
II	Sand	21.1	41.7	63.0	71.5	34.4	40.6	272.3	30.9	10.9		
III	Sand	30.7	30.2	73.0	70.1	48.2	56.3	308.5	35.4	15.4		
IV	Sand	....	53.4	102.2	68.0	48.4	8.1	280.1	33.5	13.5		
VI	Sand	....	....	32.9	65.3	45.9	62.3	206.4	11.9	-8.1		
VII	Sand	....	68.2	62.4	72.6	60.2	21.4	284.8	24.1	4.1		
VIII	Sand	....	....	....	43.2	21.3	34.6	99.1	19.4	-0.6		
Control	Sand	....	....	3.4	43.2	21.3	6.6	10.0	20.0	....		
I	Soil	52.9	55.4	97.0	61.9	61.4	50.6	379.2	18.2	15.2	1,010	461
II	Soil	24.3	52.3	22.0	74.0	45.2	48.9	266.7	41.6	38.6	797	248
III	Soil	18.6	37.9	73.6	65.7	63.7	38.1	297.6	20.4	17.4	933	384
IV	Soil	13.8	22.7	56.3	77.0	53.2	59.4	282.4	16.2	13.2	747	198
VI	Soil	21.9	53.4	78.5	70.5	46.9	56.7	327.9	16.0	13.0	823	274
VII	Soil	....	24.3	64.7	42.1	109.2	76.6	316.9	8.6	5.6	796	247
VIII	Soil	....	35.5	27.2	13.8	23.4	30.9	130.8	4.2	1.2	1,102	553
Control	Soil	....	8.5	....	16.8	13.8	5.0	44.1	3.0	....	549	...

is made soluble by treatment with 2 per cent sulfuric acid and which decomposes very readily. It is so readily decomposed by *Trichoderma* in sand culture that a considerable amount of nitrogen has to be obtained from the additional ammonium salt. In the soil, however, the decomposition of fraction VI is more rapid, and sufficient nitrogen is liberated from the other fractions by different organisms.

Portion VIII, or that part of the fungus mycelium which is dissolved by sodium hydroxide and precipitated by hydrochloric acid, gives all the characteristics of the  $\alpha$ -fraction of soil "humus." Like lignin and the  $\alpha$ -fraction itself obtained from soil, it decomposes only very slowly. When added to soil it greatly increases the "humus" content of the soil. (Table 4.) Since alka-

TABLE 5  
*Nitrogen balance of cell substance of Trichoderma*

PREPARATION	AMOUNT TAKEN OR YIELD	NITROGEN CONTENT FOR 3 GM. OF ORIGINAL MATERIAL
	mgm.	mgm.
Dry mycelium of <i>Trichoderma</i> .....	3,000	151.8
Cold NaOH extract:		
Hot HCl precipitate.....	280	20.6
Solution left.....	...	69.8
Hot NaOH extract (following the cold extraction):		
Hot HCl precipitate.....	251	4.9
Solution left.....	...	32.6
Residue, after NaOH extraction.....	987	14.4
Total nitrogen accounted for.....		142.3
Lignin content of mycelium (Schwalbe method).....	654	8.7
Lignin content of NaOH extract precipitated with hot HCl. ...	531	25.5

lies extract also certain hemicelluloses, the part of preparation VIII which has undergone decomposition, is this non-nitrogenous ingredient, as indicated by the fact that practically no ammonia is liberated as a result of its decomposition. Whereas the chitin preparation (insoluble in water, alkali and dilute acid—VII) decomposed very readily, as indicated by the evolution of CO<sub>2</sub> with the liberation of small amounts of ammonia, the "humus" preparation, or that part of the fungus mycelium which is soluble in dilute alkalies and precipitated by acids, decomposed only very slowly. Only 89.1 mgm. of CO<sub>2</sub> was given off in the sand medium and 86.7 mgm. in the soil medium, 0.6 mgm. of ammonia nitrogen was consumed in the sand medium, and 1.2 mgm. of ammonia nitrogen was liberated in the soil. This small amount of CO<sub>2</sub> is a result of decomposition of the hemicelluloses which accompanied the "humus"

fraction extracted from the fungus mycelium. In the further studies on the decomposition of the microbial protoplasm, which will be reported later, it has been found that when the "humus" fraction is boiled after precipitation with hydrochloric acid, the decomposition of this fraction by microorganisms is reduced to a minimum. In other words, the alkaline extract of fungus mycelium precipitated with hydrochloric acid and boiled in the presence of an excess of acid, gives a preparation which is identical in its resistance to decomposition to soil organic matter and to lignins.

Table 5 gives a balance of the nitrogen of the cell substance of *Trichoderma* grown on a synthetic medium, showing that most of the nitrogen is extracted by sodium hydroxide and remains in solution, after the hot hydrochloric acid precipitate is removed. "Humus"-like preparations containing varying amounts of nitrogen are obtained from this mycelium.

The results presented in this paper thus indicate definitely that the activities of microorganisms in the soil result in the synthesis of substances which possess all the characteristics of soil "humus." These substances are constant ingredients of the cells of microorganisms and are of a higher nitrogen content than the soil "humus." Their exact chemical nature remains to be determined.

The results also indicate that when plant residues are added to the soil, most of their constituents, including the monosaccharides, celluloses, and pentosans, are decomposed with comparative rapidity, although some resist decomposition. The microorganisms decomposing the various plant constituents may synthesize microbial protoplasm equivalent in weight to 20 to 30 per cent of the plant materials decomposed. This protoplasm can be further decomposed, especially in normal soils; however, a part of it resists decomposition. This constituent or group of constituents of the microbial protoplasm, a part of which is soluble in alkalis and precipitated by hydrochloric acid, contributes definitely to the soil "humus" or soil organic matter, which resists decomposition and may persist in the soil for a considerable period of time. This synthesized microbial protoplasm is probably the important source of the nitrogen found in the soil "humus."

Soil "humus," or that part of the soil organic matter which is soluble in alkalis, is thus found to be made up of substances originating from two different sources: 1. Lignins and other constituents of plants and plant residues. 2. Certain ingredients of the protoplasm synthesized by soil microorganisms.

#### GENERAL SUMMARY

A careful search of the extensive literature on the nature of soil organic matter or "humus" reveals two tendencies: 1. To describe "humus" and the so-called "humic acids" as definite compounds, the composition of which can be readily ascertained by analysis, as in the work of Berzelius, Berthelot, Oden and others; 2. To consider "humus" in the light of colloidal complexes whereby all reactions, in which soil organic matter is involved, are ascribed to

adsorption phenomena, as in the work of van Bemmelen, Baumann, and others. In both instances, the dynamic condition of the soil organic matter was not taken into consideration, and "humus" was looked upon as a mass of dead debris, originating by some obscure processes. The recent investigations of Trusov, Fischer and others, have definitely established the fact, suggested by various workers previously, that the lignins of the various constituents of natural organic matter are most resistant to decomposition and contribute largely to the soil "humus." However, these contributions as well fail to account for all the properties of the soil organic matter, the chief of which is (a) the presence of a definite amount of nitrogen in the "humus," and (b) the fact that, under certain conditions, "humus" accumulates, as in peat bogs, whereas under other conditions, "humus" tends to decompose readily, as in well aerated and limed soils.

The terms "humus," "humic acid," and other designations given to various preparations of soil organic matter have no justification either biologically or chemically. Only in popular usage can the complex soil organic matter, in contradistinction to the natural organic matter, be spoken of as soil "humus," meaning those amorphous dark-brown substances which are characteristic of soils rich in organic matter. To speak of the degree of "humification" or change in color of the organic matter in the soil as a result of various unknown agencies is merely to use terms which do not stand for any well understood processes and will no more serve to advance our knowledge of the subject in question than putting a label upon an unknown phenomenon and believing that it has thus been solved.

A study of the nature of soil organic matter or "humus" involves a number of complex problems, dealing with (a) the origin of this organic matter, (b) its chemical nature and its rôle in the soil processes and (c) its decomposition in the soil. The preliminary experiments reported in these contributions tend to throw light upon the first two problems, and a further detailed study of these as well as of the third problem is now in progress and the results will be reported later. From these preliminary studies the following conclusions may be drawn:

1. By the use of dilute sodium hydroxide, the soil organic matter can be separated into four distinct groups: (a) the part insoluble in dilute alkali, even under pressure; (b) the part soluble in alkali and precipitated by an excess of hot hydrochloric acid; (c) the part soluble in alkali and in acid, but precipitated at a definite isoelectric point, namely at pH 4.8 to 5.0, an organic-inorganic complex; (d) the part made soluble in water, as a result of treatment of the soil with the alkali. The third fraction may be absent altogether in peat soils, but it is present abundantly in mineral soils, consisting of about 50 to 70 per cent aluminum and other bases and 25 to 40 per cent organic matter. The presence and absence of this fraction allows a differentiation between peat and mineral soils. The second fraction, equivalent to the so-called "humic acids," is practically free from ash and contains from 2 to 4 per cent nitrogen.

2. When organic matter is added to the soil, the various sugars, starches, hemicelluloses, celluloses, and proteins are rapidly decomposed. The lignins and, to a lesser extent, the fat and waxes resist decomposition under anaerobic conditions, as in peat soils, and are slowly decomposed under aerobic conditions by certain groups of organisms.

3. Pure or mixed cultures of soil fungi and bacteria rapidly attack the various constituents of the natural organic materials added to the soil, converting a part of the carbon into microbial protoplasm. A definite amount of nitrogen is thereby assimilated and changed from an inorganic into an organic form or from plant proteins into microbial protoplasm. A part of this protoplasm gives all the reactions characteristic of "humus" and is rather rich in nitrogen.

4. The soil "humus" or that part of the soil organic matter which gives to it its dark color and which is more or less resistant to decomposition, is a result of the accumulation of substances of plant origin—on the one hand, largely the lignins and to some extent the fats and waxes and perhaps certain nitrogenous substances, and on the other hand, substances synthesized by microorganisms, nitrogenous in nature.<sup>2</sup>

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<sup>2</sup> The author is indebted to Mr. R. Dubos of this laboratory for assistance in carrying out the various cellulose determinations. The method used for determining the cellulose content of straw will be described in detail later.

# THE RELATION OF MANGANESE AND IRON TO A LIME-INDUCED CHLOROSIS<sup>1</sup>

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## INTRODUCTION

A chlorotic condition has been noted frequently with various crops—particularly oats, spinach, lettuce, corn, beets, and beans (4)—on soils limed to the neutral point on the experimental plats of this station. Such lime-induced chlorosis of field and garden crops, which is not a new thing, has usually been attributed to an iron deficiency. Applications of soluble iron compounds have usually been found to cure it (3), but repeated attempts to relate the condition found at this station to an iron deficiency, or to correct it by supplying iron in various ways, have failed completely. Very small applications of manganese compounds have alleviated the malady, and its occurrence is found to be associated with a very low manganese content of the affected plants as compared with that of normal plants on similar but more acid soils.

## IRON AND MAGNESIUM INEFFECTIVE AS CORRECTIVES

The chlorotic crops from the heavily-limed plats have been analyzed repeatedly for iron, with conflicting results. Oats showed marked chlorosis on the heavily-limed plat of a series of varying acidity in 1917. Analyses were made of the good oats on the more acid plats and both of the good and of the chlorotic oats on the less acid plat, with the results given in table 1.

The iron content of the chlorotic plants was found to be less than that of the green plants on the same plat (29) but both of these had a much higher iron content than those from the more acid plat (23).

Similar chlorotic conditions were observed with beans growing on certain of the plats with different kinds of lime. Chlorosis was particularly severe on plat 74, which had been heavily limed with high-calcium hydroxide, and on plat 80, which had been equally heavily limed with high-magnesium hydroxide. The beans on the control plat (82) which had not been limed, showed no signs of chlorosis.

Samples of leaves from the chlorotic and non-chlorotic plants were gathered in duplicate by two different people and analyzed for calcium oxide (CaO), magnesium oxide (MgO), and iron content, with the results given in table 2.

<sup>1</sup> Contribution 330 of the Rhode Island Agricultural Experiment Station, Kingston.



The green, healthy plants had a lower content of both iron and magnesium than the chlorotic plants; therefore the abnormal condition cannot be attributed in this case to a deficiency of either iron or magnesium (2).

In 1919, on the Phosphate Experiment plats, receiving different treatments as regards calcium oxide (CaO) and phosphoric acid ( $P_2O_5$ ), similar chlorotic

TABLE 1  
*Analysis of spring oats, 1919*

PLAT NUMBER	CONDITION OF OATS	Fe CONTENT ON DRY MATTER BASIS	CaO REQUIREMENT*
		<i>p.p.m.</i>	<i>pounds</i>
23	Good	227	5,482
25	Good	...	4,228
27	Good	907	4,452
29	Good and green	676	3,046
29	Chlorotic	475	3,046

\* Upper 12 inches of soil by the Ammonia Method (5).

TABLE 2  
*Average calcium, magnesium, and iron content of chlorotic and non-chlorotic leaves of beans, 1917*

PLAT NUMBER	TREATMENT	CONDITION OF		CaO in DRY MATTER	MgO in DRY MATTER	Fe in DRY MATTER
		Leaves	Soil			
				<i>per cent</i>	<i>per cent</i>	<i>p.p.m.</i>
74	High-calcium hydroxide	Chlorotic	Neutral	4.9	1.0	59.3
80	High-magnesium hydroxide	Chlorotic	Neutral	4.3	2.1	51.9
82	No lime	Green	Acid	3.4	0.8	37.9

TABLE 3  
*Iron content of oats and wheat, 1919*

PLAT NUMBER	TREATMENT		CONDITION OF PLANTS	Fe CONTENT ON DRY MATTER BASIS	
	$P_2O_5$	CaO		Oats	Wheat
				<i>p.p.m.</i>	<i>p.p.m.</i>
58	Ground bone	Little	Green	780	...
68	None	Little	Green	340	480
53	Acid phosphate	Much	.....	900	890
57	Ground bone	Much	Chlorotic	440	440

effects were observed on oats and wheat, and sample plants were again analyzed (table 3).

The iron content of the chlorotic plants on the highly-limed bone plat was low, but not so low in the case of oats and not materially lower in the case of wheat than of the green plants on the acid plat (68).

In the spring of 1922, spinach was found to be chlorotic on the heavily-

limed, north end of plat 128, and healthy on the lesser-limed, south end. The iron content of the spinach from 128N was 1232 p.p.m., and the pH was 7.0; and the iron content of the spinach from 128S, 815 p.p.m. with pH 6.0. The lesser-limed, healthy spinach had a lower iron content than the chlorotic spinach from the heavily-limed plat, the reaction of which was neutral.

In the spring of 1921, the plats given in table 3 were again in oats and comparisons were made of the acidities of the plats on which the plants were chlorotic or healthy. (Table 4.)

Here the relation to acidity is clear; the plats that were neutral being chlorotic, and the acid plats healthy. The oats on the neutral soils yielded less than a half crop.

Similar chlorotic symptoms had been noted previously on highly-limed plats, with corn and other crops. In 1922, these Phosphate Experiment plats were planted to carrots, corn, mangels, oats, onions, and potatoes. Of these only

TABLE 4  
*Soil acidity and condition of oats, May, 1921*

PLAT NUMBER	TREATMENT		CONDITION OF PLANTS	SOIL REACTION
	P <sub>2</sub> O <sub>5</sub>	CaO		
57	Ground bone	High	Badly chlorotic	pH 7.5
58	Ground bone	Low	Green	6.8
59N*	Thomas slag	High	Chlorotic	7.7
59S†	Thomas slag	Less	Green	6.8
60N*	Thomas slag	Little	Green	6.0
60S†	Thomas slag	None	Green	5.4

\* North half.

† South half.

the mangels and onions yielded more on the higher-limed than on the lesser-limed areas. Most of the other crops were chlorotic on the limed areas. This was especially true of corn, and injections with a variety of iron compounds in solution were made with negative results on the chlorotic corn plants.

Chlorosis of the crops on the heavily-limed plats continued to be noted in 1923 and 1924, and in 1924 this was even observed in a slightly acid soil which had previously been limed to neutrality. Thus the previous treatment appeared to have so affected the soil as to continue the chlorotic condition of the crops even for a time after the soil had become acid.

The previous experiments having yielded only negative results in an attempt to cure this lime-induced chlorosis, tests were planned for 1925 to ascertain whether ferrous iron or reducing agents which might convert ferric into ferrous iron would prove beneficial. The spinach on the market garden area having become chlorotic, on May 5, when the plants were a month old, the following

treatments (8) were tried on the odd-numbered rows of plat 101, the even ones having been left as controls:

Spraying with tap water; sulfuric acid 0.0001*M*; mono-potassium phosphate 2 per cent; ammonium nitrate 0.08 per cent; potassium sulfate 1 per cent; ammonium citrate 0.001*M*; acidulated with citric acid; ferric ammonium citrate (0.02 per cent ferric citrate and ammonium hydroxide to pH 6.5); ferrous sulfate 0.02 per cent; manganous sulfate 0.004 per cent; formaldehyde 0.04 per cent, and iron powder sprinkled along the row. (Unless otherwise specified in this paper reference is made to c. p. crystalline salts.) Thus each of the principal fertilizer ingredients was tried; also iron as citrate and as ferrous sulfate. Manganous sulfate ( $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ ) and formalin were used because they are reducing agents and might tend to convert ferric iron already present in the soil to the ferrous state.

In some instances the spraying of ferrous sulfate on the leaves, as of pineapples (6), subjected to too much manganese, has been found effective in curing an iron deficiency where applications to the soil have proved ineffective. Accordingly, the leaves of a few plants were injected with ferric ammonium citrate 0.001*M*, and ferrous sulfate 0.004*M* (about 1 per cent solution). The citrate killed the leaves, and the ferrous sulfate produced no visible effect, the leaves remaining chlorotic. The spray applications likewise gave negative results, except in the case of the manganous sulfate. The plants sprayed with manganous sulfate regained a normal green color and resumed active growth 4 days after treatment, whereas the remainder of the spinach on the plat was chlorotic.

Applications of commercial iron sulfate at the rate of about 200 pounds per acre were also made to areas of chlorotic spinach and oats on plats which had been limed to the neutral point. These likewise produced no visible effect.

The applications of iron to the soil, sprayed on the plants, and injected into the plants, have given only negative results with the lime-induced chlorosis experienced at this station. Further, the percentage iron content of the healthy plants on acid soils is not generally greater, and is frequently less, than that of the chlorotic plants. This lime-induced chlorosis seems not to be due to an iron deficiency. The chlorotic symptoms appear whether the soil is limed with high-magnesium or high-calcium lime, so the difficulty cannot be ascribed to a magnesium deficiency (2).

#### MANGANESE AS A CURE

Manganous sulfate was found to cure very promptly the chlorosis in spinach (7), in oats, in beets, and in beans on the nearly-neutral soils. Later, manganous chloride and potassium permanganate were used with the same sort of benefit. Extremely small amounts of manganese applied as sprays to the plants and soil, were found to be effective.

## ANALYTICAL METHODS

Chemical determinations of the manganese content were made on both tissue and plant solution. Fresh plants were thoroughly macerated in a Nixtamel mill and the solution was filtered through fine-mesh silk. The solution was then decolorized by shaking with carbon black and heating to coagulation. After filtering and making up the solution to volume, aliquots were freed from organic matter by the wet combustion method using concentrated sulfuric and nitric acids. Then the color was developed by the use of the periodate method of oxidation (9) and the comparisons were made against standard potassium permanganate in a Kennicott-Sargent colorimeter. For the determinations of total manganese in plant tissue, the roots and tops of the plants were separated, carefully washed free from adhering soil particles, dried, and powdered. A 4- to 5-gm. sample was ashed in a platinum dish and the ash fused with 10 to 15 gm. of potassium bisulfate. After cooling, the dish and contents were immersed in dilute sulfuric acid and the whole heated to boiling to insure complete disintegration of the fused mass. The mixture was cooled and filtered through an asbestos Gooch crucible, the filter washed free from acid, and the filtrate transferred to a beaker for oxidation. The oxidation and colorimetric determinations were carried out as outlined above with the plant solution.<sup>2</sup>

The determinations of soil pH were made electrometrically and the "lime requirement" was determined according to the modified Jones' calcium-acetate method (1).

## EXPERIMENTAL DATA

Strong solutions of iron salts injected into the tissues killed portions of the leaves, weaker solutions having no visible effect. Injections of manganous sulfate solutions into the tissues were beneficial.

On May 11, 1925, 6 days after treatment, the plants which had been sprayed with the manganous sulfate had become darker green and had started active growth. Those given the other treatments were more chlorotic than before, and were indistinguishable in appearance from the remainder of the plot.

On May 11, manganous sulfate solution was sprayed at the rate of 0.3 pound per acre and 0.15 pound per acre on two separate areas of approximately 120 square feet of plot 101.

On May 15, 4 days after these applications, the area treated with 0.3 pound per acre was already green and recovering from chlorosis, whereas the areas treated with 0.15 pound per acre showed no apparent benefit.

On May 14, applications of ferrous sulfate 0.001*M*, manganous sulfate 0.0002*M*, and potassium permanganate 0.0002*M* were made at the rate of 17 liters per 15 feet of row (in the case of manganous sulfate this amounted to

<sup>2</sup> The authors are indebted to J. S. McHargue for the suggestion of the method for the determination of total manganese.

about 1 pound of manganese per acre) on chlorotic spinach growing on the heavily-limed, neutral soil of plat 74S. Four days later, the plants treated with both the potassium permanganate and the manganous sulfate were observed to be regaining a good deep green color. The benefit was more evident with the manganous sulfate than with the potassium permanganate.

On May 23, a portion of the chlorotic spinach on each of the plats, 74, 76, 78 and 80, which were nearly neutral, and on 82 which was similarly fertilized but unlimed and strongly acid, was sprayed with manganous sulfate at the rate of about 0.5 pound per acre of manganese. This treatment was repeated on May 28, except on the acid plat 82, on which the spinach was already nearly dead because of aluminum toxicity and which at no time had shown any symptoms of the typical lime-induced chlorosis.

On June 3, each of these treated areas on the nearly-neutral plats showed uniformly green and good spinach, far superior to that on the untreated areas, which was very yellow, with large dead spots in the leaves.

TABLE 5  
*Comparative yields of spring spinach with and without manganese treatment, 1925*

PLAT NUMBER	TREATMENT	NORTHWEST QUARTER, 1 POUND Mn PER ACRE AS MnSO <sub>4</sub> ·4H <sub>2</sub> O	SOUTHEAST QUARTER, 1 POUND Mn PER ACRE AS KMnO <sub>4</sub>	NORTHEAST QUARTER, NO TREATMENT	SOUTHWEST QUARTER, NO TREATMENT
74	High-calcium hydroxide, extra lime	42.5	25.5	15.5	24.5
76	High-magnesium limestone	56.7	63.5	46.5	59.0
78	High-calcium limestone	67.0	34.0	36.5	36.0
80	High-magnesium hydroxide	56.5	37.5	19.0	29.5
82	No lime	0	0	0	0
Average increase due to treatment, <i>per cent.</i> .....		67	20		

On June 11, this area of spinach was harvested. The yields in pounds, green weight, are given in table 5.

The manganous sulfate was much more effective than the potassium permanganate in curing the chlorotic condition. Not only was the yield of spinach increased 67 per cent by the manganous sulfate treatment, but the spinach from the treated area was bright green and of good quality, whereas that from the untreated areas was so poor that it was unsalable.

The manganese content of the plant solution of the untreated plants was less than ten-millionths per cent and was not measurable. The manganese content of the whole plants at harvest was very small, but reflected quite clearly the treatment given. (Table 6.)

The manganese content of the plants was increased by the addition of manganese to the soil. The plants on somewhat acid soils (plats 29 and 53) had a

higher manganese content than those which were chlorotic (plat 76), although none of these three lots had been supplied with extra manganese.

The results with the manganese treatment of chlorotic spinach on nearly-neutral soils having been uniformly successful, a similar treatment with manganoous chloride was employed in the fall on large areas to obtain more yield data. On September 18, 22 days after planting, 8 pounds of manganoous chlo-

TABLE 6  
*Manganese content and condition of crop of fall spinach*

PLAT NUMBER	TREATMENT		CONDITION OF CROP	YIELD PER ACRE	Mn IN DRY MATTER	Mn REMOVED PER ACRE	SOIL REAC- TION
	Fertilizer	1 pound Mn as					
76	High-magnesium limestone	None	Chlorotic	tons 9.92	p.p.m. 23.3	pounds 0.037	pH 7.0
76	High-magnesium limestone	MnSO <sub>4</sub> ·4H <sub>2</sub> O	Good	12.10	34.2	0.067	7.0
76	High-magnesium limestone	KMnO <sub>4</sub>	Good	13.91	60.0	0.125	7.0
29	Chemicals	None	Good	9.73	53.6	0.100	5.7
53M.G.*	Chemicals and manure	None	Fair	6.26	54.8	0.069	7.3

\* M.G. = market garden plat.

TABLE 7  
*Fertilization of fall spinach on market garden plats*

PLAT NUMBER	TREATMENT	
	Manure	Chemicals
	tons	
74	32	None
85*	32	None
86	16	Standard + extra K
87	..	Standard + peat + extra chemicals
115	16	Standard
116	16	Standard + extra N
117	16	Standard + extra P
196†	16	Standard

\* Soil reaction, pH 7.6.

† Soil reaction, pH 6.8.

ride per acre, or approximately 2½ pounds of manganese, was applied to one-half of each of 9 plats of spinach, the other half plat remaining untreated. The plats and their fertilizer treatments are given in table 7.

On September 25, the untreated spinach on all plats except 196 was becoming very yellow, and there were brown, dead spots in the leaves. All of the areas sprayed with manganese had quite healthy dark green leaves, as had all of the

spinach on plat 196, both the treated and untreated areas being apparently alike in appearance and vigor. The spinach on these plats was harvested on October 20 to 29. Table 8 shows the yields in bushels per acre.

The yield was significantly increased by the manganese treatment on all plats except 196, on which the soil was more acid. The chlorosis difficulty seems to be associated entirely with neutral or alkaline soils. It has not appeared with any crops on soils with a pH of 6.8 or less.

TABLE 8

*Comparative yields of fall spinach with and without manganese treatment on the market garden plats, 1925*

PLAT NUMBER	UNTREATED HALF	TREATED HALF*	INCREASE DUE TO Mn
	<i>bushels†</i>	<i>bushels</i>	<i>per cent</i>
74	143	450	215
85	415	725	75
86	1,080	1,235	14
87	615	950	54
115	930	1,215	31
116	1,075	1,345	25
117	1,090	1,310	20
196	1,170	1,170	0

\* 8 pounds Mn per acre.

† One bushel of spinach equals 12 pounds green weight.

TABLE 9

*Comparative manganese content of spinach with and without treatment on the market garden plats, 1925*

PLAT NUMBER	ROTATION	TREATMENT Mn PER ACRE	H <sub>2</sub> O	Mn IN DRY MATTER	Mn IN PLANTS PER ACRE	SOIL REACTION	CaO REQUIREMENT
		<i>pounds</i>	<i>per cent</i>	<i>p. p. m.</i>	<i>pounds</i>	<i>pH</i>	
115	W	None	93.11	51.8	0.040	...	...
115	W	2	89.05	92.4	0.148	...	...
85	W	None	85.84	51.8	0.037	7.6	380
85	W	2	87.09	103.0	0.116	7.6	...
196	W	None	85.01	56.1	0.098	6.8	810
196	W	2	83.74	57.5	0.121	6.8	...

The manganese content of the harvested spinach plants from some of these plats is given in table 9.

On plat 196, where the soil was very acid, the addition of manganese at the rate of 8 pounds per acre neither improved the yield nor greatly increased the manganese content of the plants. On plats 115 and 85 the appearance of the plants and the yields of spinach were substantially improved by the manganese treatment; and the manganese content of the plants was also nearly doubled in each case. The conclusion seems reasonable that spinach requires manganese

(7), and that on these highly-limed soils there is a deficiency of available manganese which is overcome by the surface application of 8 pounds per acre in this case. That the amount required is very small is evident from the small amounts found in the healthy plants (60 p.p.m.) and the small amount of this element removed by the crop (0.1 pound per acre).

## OATS

In preliminary tests, oats which were chlorotic on heavily-limed soils, were benefited as was the spinach. Accordingly on May 28, when the oat cover crops were 54 days old and beginning to appear chlorotic, one-fourth of each of the market garden plats 77, 107, 91, 92, 121, and 122 was sprayed with manganous sulfate at the rate of 4 pounds of salt (1 pound of Mn) per acre. On June 3, six days after this treatment, the treated one-fourth of each plat was distinctly improved, having recovered a bright-green healthy color. The untreated areas showed symptoms similar to those of the spinach; the leaves became yellow, brown dead spots appeared in them, and growth was stunted.

TABLE 10

*Comparative manganese content of oats with and without treatment on the market garden plats, 1925*

PLAT NUMBER	ROTATION	TREATMENT Mn PER ACRE	H <sub>2</sub> O	Mn ON DRY MATTER BASIS	pH OF SOIL	CaO REQUIREMENT
		<i>pounds</i>	<i>per cent</i>	<i>p.p.m.</i>		
107	X	None	88.9	25.0	...	...
107	X	1	89.4	27.1	...	...
92	X	1	90.0	40.1	7.3	473

Samples of a definite area were cut from treated and untreated sections of each plat, and the green weights determined. The weight of the forage on the manganese-treated area was 67 per cent greater than on the untreated areas.

The analyses of samples of oats for manganese content are given in table 10.

With oats as with spinach, the addition of very small amounts of manganese restored the health of the plants and also increased the yields and manganese content.

The above experiments with spinach and oats clearly indicate that the lime-induced chlorosis found with them was cured by applications of manganese salts—either manganous salts or permanganate. Further, the chlorotic plants had a lower manganese content than healthy plants on more acid soils.

The apparent manganese requirement of the plants is extremely small. Expressed in per cent of the dry matter, it was between 0.002 and 0.006 per cent for oats and spinach.



## GENERAL SUMMARY

1. Chlorosis in this case is not due to a deficiency of iron, as the iron content of the chlorotic plants equalled or exceeded that of normal plants, and iron treatment was ineffective in curing the malady.

2. Chlorosis appeared on soils treated with both high-magnesium and high-calcium lime, thus indicating that a magnesium deficiency was not causing the trouble.

3. Small amounts of manganese salts cured the chlorotic condition.

4. The chlorotic plants had a lower manganese content than normal plants.

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# A CONTRIBUTION TO THE STUDY OF INTERRELATIONS BETWEEN THE TEMPERATURE OF THE SOIL AND OF THE ATMOSPHERE AND A NEW TYPE OF THERMOMETER FOR SUCH STUDY<sup>1</sup>

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The actual amount of heat reaching the surface of the earth is only that portion of the sun's rays that passes unabsorbed through the atmosphere. The moisture content of the soil has a great influence in determining the temperature of the soil because of the higher specific heat of wet soil, which is close to 1 whereas that of dry soil is approximately 0.2. The surface layer of the soil frequently is hotter than the air, especially on sunny days in hot climates. Leather (8) states that the maximum temperature at Pusa (India) is 20°C. above the maximum air temperature in the shade.

Russell (12) summarizes the factors influencing soil temperatures as follows:

(a) A south slope is warmer than a north slope.

(b) Bare land is warmer than land covered with vegetation, excepting during winter months.

(c) Soil exposed to the sun's rays is often hotter than the air, and is subject to considerable temperature variations, which, however, only slowly affect layers three or more inches deep.

(d) Moist soil, being a better conductor than dry soil, is much more uniform in temperature.

(e) The top 6 inches of soil has a higher mean temperature than the air both in summer and in winter. At 6 inches the warmer part of the day centers around 5.30 p.m., and the cooler part around 9.30 a.m.

(f) The warming of the soil in spring is facilitated by drying; the cooling in autumn is increased by clear nights and diminished by rain [Keen and Russell, (7)].

The temperature of the soil and of the atmosphere is determined by certain laws and the importance of temperature as one of the climatic elements is well illustrated in the "Climatic Laws" formulated by Visher (14) in which he gives eleven meteorological laws which relate to climate and which, he shows, refer to a cycle due to their joint action. He states

The sun rises; its rays penetrate the air but are absorbed by the dark soil which thereby is warmed and gives out long heat rays which are absorbed by the air. As the air becomes

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<sup>1</sup> Part 1 of a thesis submitted at the University of Wisconsin in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The writer wishes to express his appreciation for the helpful suggestions and criticisms tendered by Professor A. R. Whitson.

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warm it expands, winds are set up, and the heated air is moved away and warms other places, where the sun's heating is less effective. Sometimes the air rises and is therefore cooled adiabatically, occasionally enough so that its moisture is partly condensed with a liberation of heat which still further warms the air. Gradually the heat escapes outward into space or downward to cooler earth and water until the air is no longer relatively warm. Then gravity pulls it down to the earth's surface where it awaits another warming the following day.

The temperature of the air and of the soil is to a great extent beyond the control of man. The latter, however, by certain soil management methods can be slightly altered. This is climatically perhaps unimportant but practically is worthy of careful consideration (9).

In the cranberry marshes of Wisconsin it was noted by Whitson (15) in 1905 that patches of frost occurred on level land where the marshes were poorly drained or covered with weeds, grass, or moss; whereas clean, well-drained, or sanded lands often escaped. That cultivation, draining, and sanding are means of frost control in the marshes of Wisconsin was also noted by Cox (3) who further stated that because of radiation from the warm soil it is practically impossible for frost to occur in the bogs on the first cool night following a warm spell but that it is likely to occur on the second night after the soil has become cold. The lowest temperature in the marshes is usually where the vegetation is heavy and the soil poorly drained, because the soil cannot heat up during the day. When a soil is bared it becomes heated by the sun's rays according to the nature and the color of its particles and according to its humidity. Seeley (13) emphasized the fact that the temperature of a soil covered with vegetation is dependent more upon the height of the plants and their thickness over the surface of the ground than upon the kind of a plant. In a forest the loss of heat by radiation at night is checked by the forest canopy. In a comparison between parks and timbered areas, Pearson (11) found that the mean annual temperature in the forest was 2.7°F. higher than in the park, that the maximum extremes average 0.9° lower and the minimum extremes 6.4° higher and that the mean daily range was 7.3° smaller in the forest. He also showed that the average wind movement in the forest was only 51 per cent as great as in the park. Geddes (6) states that where vegetation is rich, the temperature variation during a 24-hour interval is much smaller than in a dry, barren region. In the latter, all the energy goes to warm the barren surface of the earth, and, as the air is very dry, there is nothing to prevent radiation at night. In the former, much of the energy is absorbed by the vegetation, and the moisture in the atmosphere arising from the vegetation prevents radiation at night.

Coit and Hodgson (2), in their investigations on the abnormal shedding of young fruits of the Washington Navel oranges, studied the climatic environment in several groves in California, for the purpose of obtaining an integration of all the climatic factors in their effect upon the plant. They selected the Livingston white cylindrical porous cup atmometer. They found at their desert station, which was located on the open, bare desert about one-half mile

to windward of the edge of the orchard and many miles to leeward of any irrigated land, that the atmometer lost an average of 94.0 cc. of water daily whereas at their alfalfa station the atmometer lost only 18.5 cc., or only 20 per cent as much. Thus they show within a half mile in the San Joaquin desert a climatic change of the same magnitude as that between Miami, Florida and Tucson, Arizona. The same authors state that it is possible to modify climatic conditions in an orchard so as to set crops that are in every way comparable with those produced in much more climatically favored citrus districts. The means suggested are by heavier and more frequent irrigation, the planting of intercrops, mulching with straw and other materials, protection by means of windbreaks, and a reduction of leaf areas by moderate winter pruning.

#### PLAN OF INVESTIGATION

The purpose of this investigation was to determine more clearly some of the temperature interrelations between the soil and the atmosphere. For this purpose temperature readings were taken during the day as well as during the night, especially when it was calm.

The instruments used were standardized chemical thermometers certified by the United States Bureau of Standards and checked by the author, a copper bulb thermometer devised by the author, and electrical resistance thermometers.

Certain stations were selected on the University of Wisconsin farm, located in Madison, Wisconsin and at the Branch of the College of Agriculture, University of California, Davis, California. The stations were so located that temperature readings could be taken in an uncropped area adjoining a cropped area as well as in the latter. The cropped areas selected were both on mineral soils and on organic soils at Madison and only on the former at Davis. The crops were sugar beets, hemp, corn, nursery, oak forests, cabbages, vineyard and orchard.

Air temperatures taken at various heights above the soil surface in these cropped areas when compared with those at the same heights in adjoining uncropped areas where the land was bare, showed a great difference.

The day temperatures were higher (over 6°F. at times) in areas planted to such crops as sugar beets and corn, because these crops permitted the sun's rays free access and because they reduced considerably the velocity of the wind as compared to the uncropped areas. In the case of a crop such as hemp where the stand was very thick, lower temperatures were obtained during the day at the 6- and 12-inch heights and higher temperatures at the 36- and 60-inch heights as compared to the adjoining uncropped areas because the sun's rays were able to penetrate only a short distance through the hemp whereas the height of the hemp, which was 8 feet, reduced the wind velocity so that the air temperatures in the upper portion of the hemp were higher.

Air temperatures taken before sunrise when there was a slight wind or when

it was calm were higher in the cropped areas than in the uncropped areas. In the case of hemp the air temperatures at heights of 6, 12, 36, and 60-inches were generally from 0.5 to 6.8°F. higher than in the uncropped plots.

Inversions of temperature were found to be the greatest over uncropped plots, whereas the temperatures in the cropped plots were more nearly uniform at the 6-, 12-, 36-, 60-inch heights. On the University Marsh at Madison, Wisconsin (4), an inversion of 4.8°F. was obtained between the 6- and 60-inch heights whereas at the same elevations in the hemp plot there was an inversion of only 0.5° at the same time. These inversions were found to extend to a height of 96 inches when the greatest height above the soil at which the air temperatures were taken was 168 inches.

The importance of air drainage is well shown in readings obtained in a small nursery at Madison, Wisconsin where the air at the lower end of the nursery was at all heights (6 to 60 inches) from 0.4 to 1.4° lower in temperature than at the upper end which was only three feet higher in elevation. A similar effect was found in a vineyard on the University Farm at Davis, California where there was a difference of from 1.4 to 2.7°F. between the upper station which was six feet higher in elevation than the lower station.

#### TEMPERATURE OF SURFACE SOIL

The next phase of this problem investigated was the relation between the temperature of the immediate surface of the soil and the temperature of the air in contact with it. Various references in literature note that after sunset the surface of the soil becomes cooler than the air in contact with it. One of these articles (16) makes the following comment:

After the sun goes down the ground cools rapidly through radiation, and its temperature soon falls below that of the layer of air in contact with it. As soon as this occurs the surface air begins to lose heat to the ground by conduction. The air near the ground now becomes cooler than the air above and its density becomes increasingly greater.

Franklin (5) has shown that the temperature of the surface soil depends on the relative humidity, the dryness of the surface layers, and the temperature of the underground layers. He noted that where the soil had been kept covered during the day, as for instance by a canvas shelter, except in times of cold, rain, sleet, or strong winds and closed at night, the temperature under the shelter was from 2.5 to 7°F. higher than in the open soil. The same author reported results (5) which show that if the surface soil does not freeze, the air minimum over open soil follows the surface soil minimum very closely.

Frost occurrence is more likely on organic than on mineral soils because of the difference between their effects on air temperatures. This is due to the slower rate of heat conductivity in the case of the organic soils (1), the temperatures of which at the lower depths may be higher than those of the mineral soils. The mineral soils are good conductors of heat and thus allow the heat

which has been accumulated at the various depths, during the day, to travel to the surface at a greater speed. Patten (10) has shown that the greatest factor to be considered in heat conductivity is the moisture of the soil.

Bouyoucos and McCool (1) found the surface temperature of the muck soil to be 28°F. and that of the air one inch above the soil surface to be 30.5°F. They obtained this air temperature by having the thermometer in a box similar to the United States Weather Bureau shelter but with the bottom removed. The surface of the mineral soils they reported, however, as being 2.5°F. warmer than the air one inch above.

The problem of obtaining the temperature of the immediate surface of the soil was felt by the author to be of prime importance. The methods used in the past have been either to lay the thermometer so that the bulb was on the top of the surface of the soil or to cover the bulb with a thin layer of soil.

A large number of readings by both methods were obtained by the author on mineral as well as on organic soils and in only one case was the temperature of the surface soil colder before sunrise than the temperature of the air in contact with it. This result was obtained on the peat soil, when the surface soil was moist, the air calm, no fog, stars visible, and with frost on the ground. These readings were obtained with mercury thermometers with the bulbs lying on the surface of the peat and  $\frac{1}{2}$  inch above the surface. The air temperature was 26.4°F. whereas the surface temperature was 25.7°F.

It was thought necessary to use some means other than the ordinary type of thermometer to obtain the actual temperature of the immediate surface of the soil because this temperature cannot be obtained by covering the bulb of the thermometer with a thin layer of soil nor, because of the varying thickness of the bulbs, and their length, and of the fact that the contact is just a line contact, can it be obtained by placing the thermometer on the soil surface. Because it was felt to be necessary to obtain temperatures during the same night at various stations rather distant from each other, a portable outfit was necessary. For this reason mainly it was decided to enlarge the bulb of an ordinary thermometer.

Pieces of well seasoned basswood (fig. 1) measuring 3 by 3 by  $\frac{3}{4}$  inches were used. A groove of sufficient length and depth to accommodate the bulb of a mercury thermometer was cut in each of these blocks. Dental cement was then placed in this groove, the bulb placed in the cement so that it was completely covered and then a piece of thin copper plate  $\frac{1}{16}$  of an inch in thickness was soldered to the bulb so that it was flush with the outside face of the block. By placing this copper plate on the surface of the soil it was felt an accurate record of the temperature could be obtained. Dental cement was used for making the contact between the copper plate and ordinary mercury thermometer because it is a good conductor and does not contract on hardening. These copper bulb thermometers were standardized against each other and against standard mercury thermometers by the use of cold storage rooms which were maintained at various temperatures, by placing on frozen soil

and covering the bulbs with several layers of burlap, and by placing on hot bottles and covering with burlap so that the graduated stems were exposed for reading.

The copper bulb thermometers as well as the mercury thermometers checked each other closely. The greatest difference at any temperature was  $0.6^{\circ}\text{F}$ . Corrections were made in all readings for these differences so that the temperatures reported are thought by the writer to be comparable with each other.

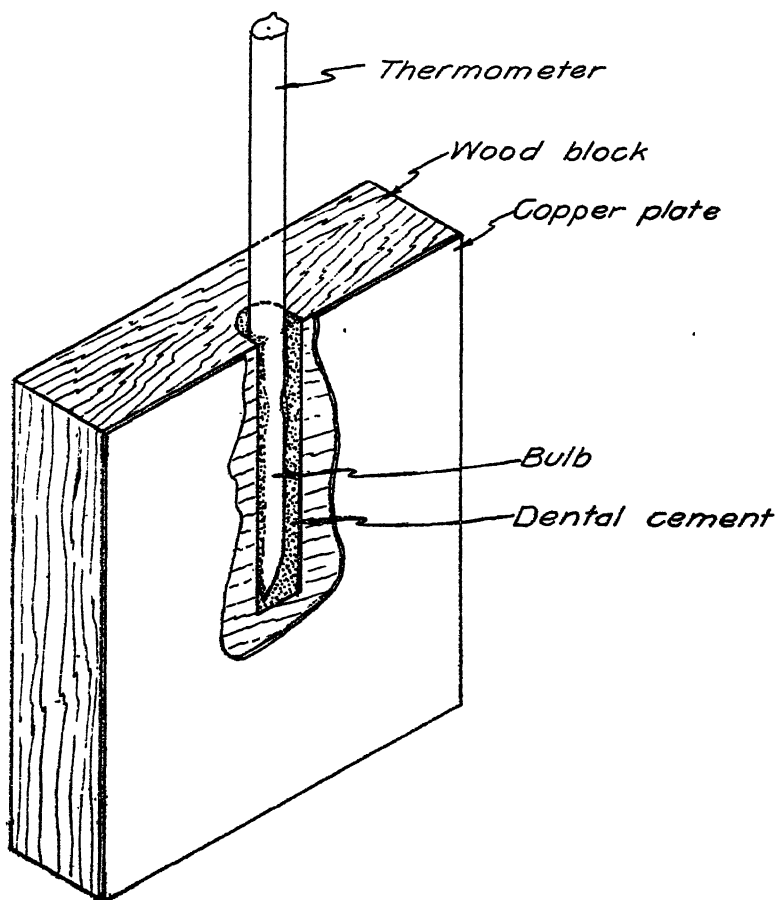


FIG. 1. COPPER BULB THERMOMETER

An attempt was made to use these copper bulb thermometers during sunlight or daylight hours but the radiation from the sky on a cloudy day was found to affect this type of thermometer so that it registered several degrees higher than the mercury thermometer. This affect of sky radiation is no doubt due to the large surface (3 by 3 inches) of the copper bulbs as com-

pared to the small area of the bulb in the mercury thermometers. Eliminating sky radiation by placing the thermometers in a tightly covered box with only the stems extending out resulted in the same temperature being obtained by the use of either type of thermometer.

The copper bulb thermometers could be used, however, during the night and were of greatest value during the hours preceding sunrise. The temperature of the surface soil was found to be lower than that of the layer of air in contact with it (one-half inch above the soil surface) just before sunrise on calm nights. During the fall of 1923 the surface of the peat soil on the marsh was seldom more than  $1.5^{\circ}$  cooler than the air in contact with it; whereas with mineral soils lying at higher elevations, a difference as high as  $4.55^{\circ}\text{F}$ .

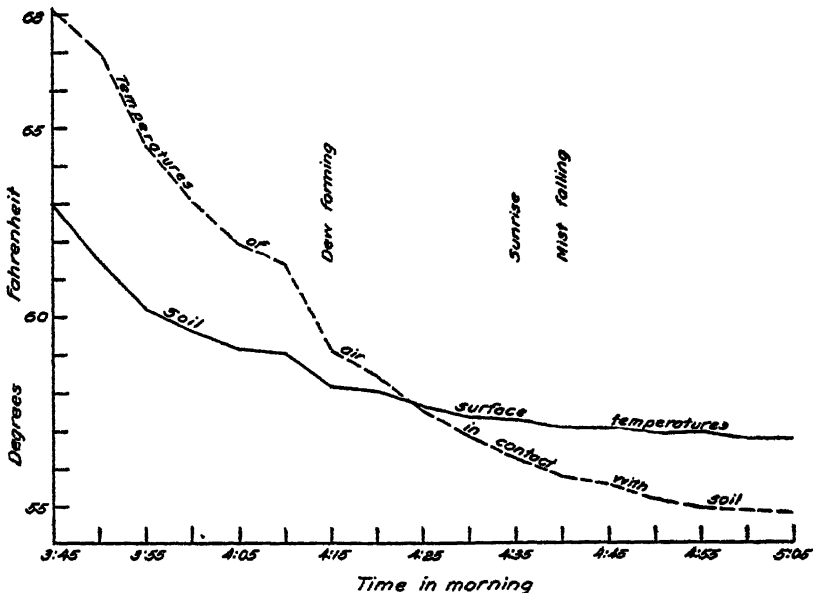


FIG. 2. TEMPERATURES AT STATION 1, JUNE 11, 1924

was obtained, especially when the temperatures were as high as  $40$  and up to  $68^{\circ}\text{F}$ . When the temperature of the air was close to freezing no great difference in temperature between the soil surface and the air in contact with it was noted.

In the spring of 1924, on calm nights the difference in temperature between the surface of the mineral soils and the air in contact with it was greater than was the case on marsh soils, especially when the humidity of the atmosphere was high. The greatest difference found in the spring of 1924 was at station 1 on a silt loam soil where the surface temperature was  $5.1^{\circ}$  lower than the air in contact with it. (Fig. 2.)



## TEMPERATURE CHANGES BELOW SOIL SURFACE

Soil temperatures taken at depths of 1, 3, and 6 inches by use of mercury thermometers, in general showed no great change during the period of observations before sunrise, which in some cases was two and one-half hours in length. The temperature of the surface soil, however, did change markedly during this period as did the air temperatures taken at various heights.

## USE OF ELECTRICAL RESISTANCE THERMOMETERS

A series of sixteen electrical resistance thermometers for obtaining soil temperatures was installed at Davis, California. The soil area selected was alluvial in origin with a loam texture 3 feet deep. The topography was nearly flat. Sixteen plots, 5 meters square were fenced in and a path 2 meters wide was left on the four sides of each plot. The electrical resistance thermometers used were of the bulb type and a sixteen-point automatic recorder was installed in a small one-story frame building with a shingled roof which was 100 feet north of the plots. The thermometers and recorder were supplied by the Leeds Northrup Company. The same amount and quality of wire was used to each thermometer from the recorder and the installation was made overhead so as not to disturb the soil. Cable and conduit were used from the recorder house to the center of five plots. The thermometers were standardized after all connections were made but before they were buried in the center of the plots and were found to be on the average within  $\frac{1}{2}^{\circ}\text{F}$ . (Plate 1.)

In burying the thermometers a small hole was dug in the center of each plot and the soil from each horizon was separately laid aside. The thermometers were pressed into the north wall of this hold so that their bulbs were in undisturbed soil. The soil was placed back in its original position and slightly tamped in order that each horizon might be put back in as near its original compactness as possible.

Sixteen resistance thermometers were placed at depths varying from 3 inches to 36 inches, one was on the soil surface and one during part of the spring of 1925 was  $\frac{1}{2}$  inch above the surface of the soil. From two to six thermometers were placed in each of the five plots.

The recorder was so adjusted that a temperature record from each thermometer was obtained every 15 minutes. The recorder was run continuously day and night except during short intervals when adjustments and repairs had to be made.

A comparison of the electrical resistance thermometers with the copper bulb and mercury thermometers on calm nights showed a difference of  $0.5^{\circ}\text{F}$ .

The temperature of the air  $\frac{1}{2}$  inch above the soil surface was never found to be more than  $1^{\circ}$  higher than that of the surface soil when the electrical resistance thermometers were used. This difference is not so great as was found at Madison by the use of the copper bulb thermometers, but because there were so few calm nights at Davis during the spring of 1925 it cannot be said

at present that the same difference will not be found at the latter place when this work is continued along related lines.

The same effect of sky radiation was found when the electrical resistance thermometers were exposed to the direct sunlight. The author has under consideration a method for the elimination of this factor.

As the thermometers were installed on February 19, 1925 no seasonal changes of temperature can be reported at this time (May 1, 1925). Changes in temperature during a 24-hour interval have in general been found up to the present to extend to a depth of 12 inches in the soil. When the maximum temperature of the surface soil and of the air in contact with it occurred at 2:10 p.m., the maximum temperature at the 12-inch depth did not occur until three hours later. When the minimum temperature of the surface soil and of the air in contact with it occurred at 4:42 and 5:50 a.m. respectively, the minimum temperature at a depth of 12 inches did not occur until five hours later.

A further study which will determine the rate of temperature changes in soils under different conditions is in progress.

#### SUMMARY

Air temperatures taken at heights varying from 6 to 60 inches above the soil surface were higher during the day (over  $6^{\circ}$  at times) in cropped areas, such as sugar beets and corn, than in uncropped areas. With a crop such as hemp, where there was a heavy stand, lower temperatures were obtained during the day at the 6- and 12-inch heights and higher temperatures at the 36- and 60-inch heights, as compared to adjoining uncropped areas.

The night air temperatures taken immediately preceding sunrise, when the atmosphere was calm, were higher in the cropped than in the uncropped areas. The greatest differences were found in the hemp, where the air temperatures were at times  $6.8^{\circ}$  higher.

Inversions of temperature were greatest over the uncropped plots. The highest ( $4.8^{\circ}$ ) was over marsh soil.

By the use of an enlarged bulb thermometer, the temperature of the surface soil was found to be lower than that of the air in contact with it just before sunrise on calm nights. Mineral soils, where the drainage conditions were good, showed differences as high as  $4.55^{\circ}$ ; whereas on poorly drained areas, such as peat soils, the temperature of surface was seldom more than  $1.5^{\circ}$  cooler than the air in contact with it.

During calm weather the temperature of the air in contact with the soil was found to be higher by the use of the copper bulb as well as by the electric resistance thermometers.

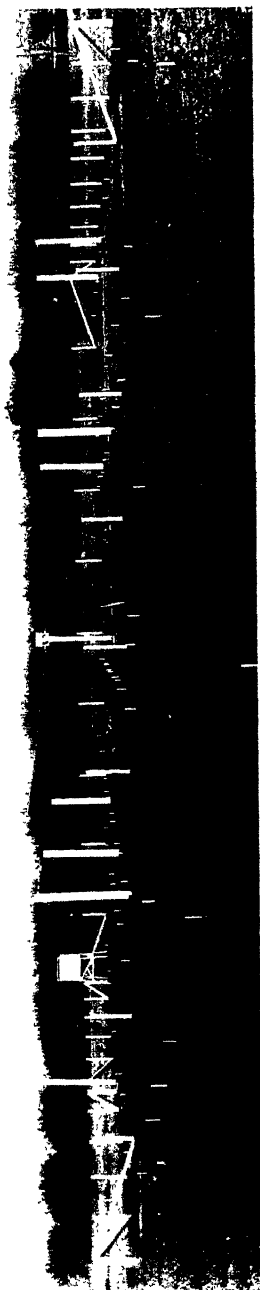
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## PLATE 1

### SOIL TEMPERATURE PLOTS AT DAVIS, CALIFORNIA





# SYNTHETIC CALCIUM SILICATES AS A SOURCE OF AGRICULTURAL LIME: III. A COMPARISON OF THE INFLUENCE OF SYNTHETIC CALCIUM SILICATES WITH OTHER FORMS OF LIME ON THE SOIL REACTION<sup>1</sup>

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## INTRODUCTION

As has been pointed out by Page (9) the researches of Bradfield (5) serve as a compliment to the theories of Hissink (7) in regard to the nature of soil acidity and its relationship to the colloidal portion of the soil. The correlation of the degree of saturation of the soil colloids, both clay and humus, and the hydrogen-ion concentration of the soil suspensions has, therefore, meant a distinct advancement in the study of soil acidity. Wiegner (13) has shown that we may consider hydrogen as the general ion adsorptively held on clay particles. From the studies of Ramann (11) on finely divided quartz and on permutites, and from studies by Bradfield (4) on clay suspensions, we may assume that the addition of bases to the soil as an agricultural practice is primarily a neutralization process with some exchange action.

As a natural outgrowth of the studies quoted above, a correlation between the pH of the soil suspensions and so-called "lime requirements" of the soil may be expected within only a given type of soil and even then imperfectly because of the influence of humus. Thus the classification of soils according to the size of particles and the humus content assumes an added significance in the recommendations of the soil scientist to farmers. Again, the measurement of the hydrogen-ion concentration of the soil has only qualitative significance unless one assembles many other data relative to the soil, i.e. content of clay, humus, exchangeable bases,  $\text{CaCO}_3$ . Although methods based on the physico-chemical properties of the soil are as yet far from perfection, the service rendered by Hissink, Gedroiz and others shows the importance of such studies and points to the ultimate significance which they will no doubt assume in the study of the soil.

<sup>1</sup> Part III of a dissertation presented to Rutgers University by R. M. Barnette in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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Barnette (1, 2) among other investigators, has shown that the ultimate effects on plant growth and on bacterial processes of different liming materials are essentially the same when the materials are added on a chemically equivalent basis. The present study was made during the process of the experiments referred to above and shows the effect of several liming materials, especially synthetic calcium silicates, on the hydrogen-ion concentrations of soil suspensions.

#### EXPERIMENTAL RESULTS

The method of Gillespie (6) and the apparatus of van Alstine (12) were used in determining the pH of the soil suspensions. The suspensions, made up of 1 part of air-dried soil to 2.5 parts of water, were shaken thoroughly and allowed to stand over night. The hydrogen-ion concentration was determined in the clear or slightly turbid liquid drawn from the top of the settling suspensions. Although it is appreciated that this method is open to objections,

TABLE 1  
*Average pH values for the suspensions of the variously treated soils\**

TREATMENT	SOIL FROM PLOT N	SOIL FROM PLOT 11A
No lime.....	5.74	5.59
Ground limestone.....	6.85	6.55
Di-calcium silicate.....	7.02	6.61
Hydrated lime.....	6.88	6.39

\* Liming materials on basis of 2000 pounds CaO per acre. All values are the average of seven samples.

it is felt that in the loam soils the buffer action is probably sufficient to prevent any great change in the suspensions through standing over night.

Soils from plots N and 11 A of the New Jersey plots were used in the initial experiments.<sup>3</sup> They were treated with ground limestone, di-calcium silicate, and hydrated lime on an equivalent lime basis of 2000 pounds of CaO per acre and maintained at a constant moisture content, uncropped. Representative samples were taken at intervals. The experiment was carried out in pots under conditions which precluded leaching.

Table 1 gives the average pH values for the suspensions of the variously treated soils. Figure 1 gives the average changes in pH values of suspensions of the variously treated soils as compared with the original soils. The values for the soils receiving the different materials on an equivalent lime basis were near enough to come well within the limits of error of this type of experimentation. The time intervals were 1 week, 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, and 13 weeks after the date of application.

From these results it may be seen that the different liming materials affected

<sup>3</sup> These soils are described in a previous publication (1).

the hydrogen-ion concentration of the soil suspensions in much the same manner. The hydrogen-ion concentrations of the soil suspensions showed a progressive increase with time (fig. 1), the changes were similar for all the different materials, and the pH values were practically the same for the various time intervals and for the three materials. The curves in figure 1 are thus typical of the changes produced by the prolonged contact of the soil with a liming material. The development of carbonates, nitrates, and sulfates, together with the fixing processes, is responsible for these changes. These

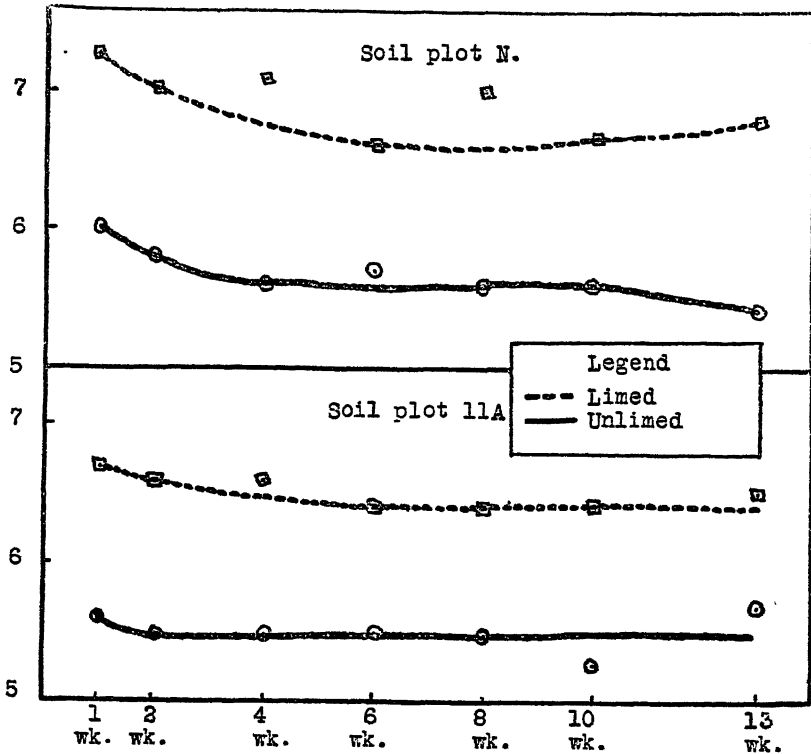


FIG. 1. CHANGE IN HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS OF LIMED AND UNLIMED SOILS WITH TIME

results are in accord with the findings of Hoagland and Christie (8). More recently Bobko and Druschinin (3) have shown that the buffer system in extracts of humus-free limed soils (soils containing  $\text{CaCO}_3$ ) is essentially the system: free  $\text{CO}_2$ - $\text{HCO}_3$ ; whereas in those of unlimed soils, "podsoils," humus-rich soils, and "torfs" the system is controlled by an acid having a larger dissociation constant than carbonic acid.

Soils from plot 11 A, from an acid Sassafras loam, and from a well limed Penn loam were used in obtaining the results given in table 2. The same



methods were employed as above and the soils were treated similarly save that they were cropped. From this table and from figure 2, the influence of increasing amounts of lime as "limosil" on the hydrogen-ion concentration of soil suspensions may be seen. The well-limed Penn loam soil showed little change of hydrogen-ion concentration with the addition of increasing amounts of "limosil." The buffer action in this soil was exceptionally pronounced. When it is recalled that "limosil" is a mixture (calcium silicate, for the greater part, and lime), it may well be imagined that the maximum solubility of the compounds at the  $\text{CO}_2$  tension present was reached with the smallest application with the resulting more or less constant pH (5). In the acid soils (plot

TABLE 2

*The influence of applications of the various liming materials on the hydrogen-ion concentration of three soils under crops*

TREATMENT	EQUIVALENT APPLI- CATION OF $\text{CaO}$ PER ACRE	SOIL FROM PLOT 11A					SASSAFRAS LOAM					PENN LOAM				
		After 1 month	2 months	8 months	11 months	Average	After 1 month	2 months	8 months	11 months	Average	After 1 month	2 months	8 months	11 months	Average
	pounds	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH	pH
No lime.....		4.85	4.9	4.8	4.05	4.65	5.2	5.95	5.45	4.45	5.26	7.05	6.95	6.6	6.4	6.75
	500	6.10	5.3	5.0	5.3	5.42	6.7	6.6	5.9	5.5	6.17	7.1	7.1	6.4	6.7	6.83
Limosil.....	1,000	6.8	5.9	5.5	5.3	5.75	6.8	6.2	5.7	5.8	6.12	7.3	6.9	6.7	6.8	6.93
	2,000	6.5	6.2	5.8	5.85	6.09	6.9	6.5	5.95	6.05	6.35	7.25	7.4	7.4	6.9	7.24
	4,000	6.9	6.7	6.3	6.4	6.57	7.2	6.7	6.2	6.5	6.65	7.3	7.3	7.5	7.2	7.32
Ground lime- stone.....	2,000	6.3	6.6	5.9	5.9	6.17	7.2	6.5	6.0	6.1	6.45	7.1	7.3	7.0	6.7	7.03
Hydrated lime.....	2,000	6.5	6.1	5.5	5.9	6.00	6.8	6.2	6.0	5.9	6.23	7.3	7.3	7.4	6.8	7.20
Di-calcium silicate.....	2,000	6.4	6.6	6.1	5.9	6.25	6.8	6.5	6.3	5.9	6.38	7.1	7.1	7.4	6.9	7.13

11 A and Sassafras loam) an entirely different condition prevails; here the soils exhibit the typical buffer curves of acid loam soils.

The four liming materials, "limosil," ground limestone, hydrated lime, and di-calcium silicate, applied on a chemically equivalent lime basis (2000 pounds  $\text{CaO}$  per acre) produced substantially equal changes in the hydrogen-ion concentration of the soil suspensions. The pH values for the different soils naturally varied.

The change of the hydrogen-ion concentration with time of contact of the materials is similar to that observed in the first series; the limed Penn loam showed the least increase. Figure 3 gives the average results for the three soils treated with the four different materials on an equivalent lime basis. The gradual decrease with elapse of time is noted.

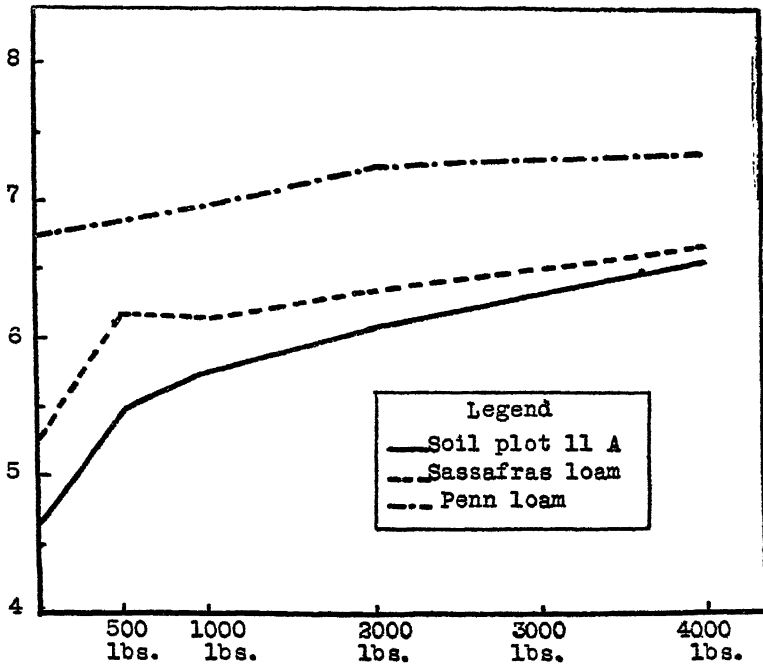


FIG. 2. RELATIONSHIP BETWEEN INCREASING APPLICATION OF LIME AS "LIMOSIL" AND HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS

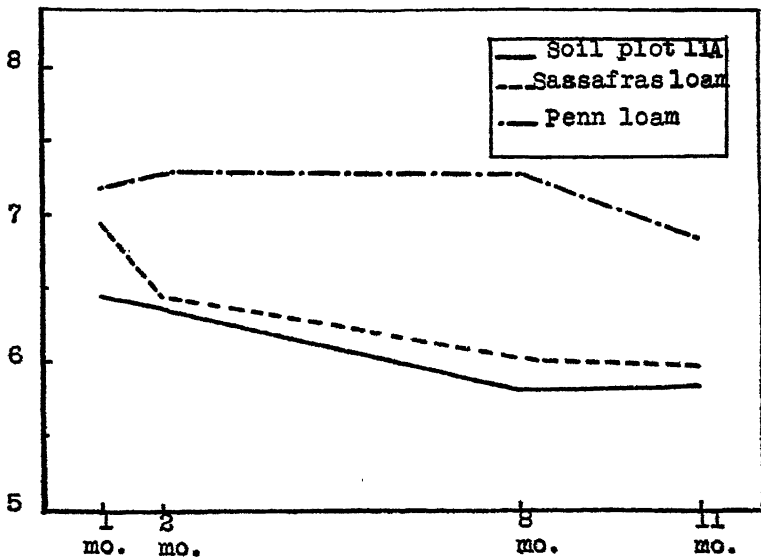


FIG. 3. CHANGE OF HYDROGEN-ION CONCENTRATION OF SOIL SUSPENSIONS TREATED WITH VARIOUS LIMING MATERIALS ON A SIMILAR CaO BASIS

In order to determine the influence of cropping on the hydrogen-ion concentration of soil suspensions a series of pots was started in which one set was treated with five different liming materials on an equivalent lime basis and cropped, and in which the other set was left uncropped. Samples of the soil were taken at intervals and the pH values determined by the colorimetric method. Samples were taken at 4-week intervals after the second week, ending with the twenty-fifth week. The Sassafras loam used in the experiments above, and an acid Elkton silt loam were used. These soils were cropped first to buckwheat and then to soybeans. The averaged pH values for the seven samples of the cropped and of the uncropped series taken at various intervals are given in table 3.

From this series it may be assumed that there is no definite influence on the hydrogen-ion concentration of soil suspensions due to cropping. This is in accordance with the findings of Pierre (10).

TABLE 3  
*The effect of cropping on the pH values of soil suspensions*

TREATMENT	WATER EXTRACT OF SASSAFRAS LOAM*		WATER EXTRACT OF ELKTON SILT LOAM†	
	Cropped	Uncropped	Cropped	Uncropped
	pH	pH	pH	pH
No lime.....	4.91	4.81	4.79	4.71
Calcium oxide.....	6.17	5.94	5.76	5.99
Hydrated lime.....	6.11	6.04	5.83	5.89
Ground limestone.....	6.07	5.85	5.70	5.89
Di-calcium silicate.....	6.17	5.97	5.81	6.41
"Limosil".....	6.20	6.18	5.80	6.11
CaO $\rightleftharpoons$ free CaO in limosil.....	5.10	4.99	4.95	5.11

\* Liming materials on the basis of 2000 pounds CaO per acre.

† Liming materials on the basis of 3200 pounds CaO per acre.

#### GENERAL DISCUSSION

The changes in the soil system brought about by the addition of a liming material are so complex that an extensive comparison of the different materials is seldom made. An investigator must limit his work to one material in order to gather enough data conclusively to defend his findings. Thus on the system: acid soil + ground limestone, we find many studies with regard to the influence of the properties of the limestone and of its fineness of divisions, which together with the action in various types of soil assist in clarifying the general problem. One may conclude from numerous similar studies that although the initial effects of the different liming materials, when applied on a practical basis to soils deficient in lime, may be different, the final effects are the same whether the lime is applied as carbonate, hydrate, or silicate so long as the application is practical. Naturally, such a statement applies only

to materials which are finely enough divided to give a comparable surface action.

Thus, studies with plants, with microbiological processes, and with the reaction of acid soil indicate that when the different liming materials are applied on a chemically equivalent and a practical basis, final reaction with the soil is the same. The researches of MacIntire and his associates, together with numerous other workers show that a formation of silicates and a combination with humus of basic materials take place. It must be recalled also that the formation of carbonates, of sulfates, and of nitrates in the soil is apparently dependent upon the available base (Ca, Mg, etc.) in a soil. From this viewpoint, the synthetic calcium silicates studied are not different in their action from other common liming materials.

#### SUMMARY

From studies of the hydrogen-ion concentration of soils treated with different liming materials, the following observations were made:

1. Chemically equivalent and practical applications of calcium carbonate, hydrated lime, dicalcium silicate, and "limosil" (mixture of mono-calcium silicate and CaO) produced, within the limits of experimental error, equal changes in the hydrogen-ion concentrations of soils to which they were added.
2. There is a progressive increase in the hydrogen-ion concentration of soils treated with liming materials following the date of application.
3. The measurement of the pH values for two acid loam soils treated with increasing applications of "limosil" showed typical buffer curves for loam soils; whereas the pH values for a well limed Penn loam (free from humus) showed little increase with increased applications of "limosil."

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# A STUDY OF THE ROOT-NODULE BACTERIA OF WOOD'S CLOVER (*DALEA ALOPECUROIDES*)<sup>1</sup>

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Wood's clover is becoming recognized as a valuable annual legume for use as a green manure. Since it is a newly cultivated legume, the question of the relation of its root-nodule bacteria to the already recognized groups of nodule bacteria is of decided importance. Schneider (2) reported in 1892 that the bacteria from nodules of *Dalea* were different from the others, which included the bacteria of many common legumes, then under study.

## CROSS-INOCULATION STUDIES

In order to place in the proper group the bacteria of a legume not previously studied, it is necessary to make cross-inoculation tests with typical members of the established groups. No other method of placing an unknown organism in a group has yet been devised. It is evident that the cultures used for such experiments must be free from all root-nodule bacteria except those common to the legume or group to be studied. The presence of only a few organisms of any other legume in the culture applied would give erroneous results in such crosses.

The cross-inoculation results reported in table 1 were obtained from seven experiments in which three to five jars, containing from 100 to 200 plants per jar, were used for each strain of the organism tested.

Wood's clover nodule bacteria have not been found to cross inoculate with the eleven groups represented in these experiments. From these results there has been established a new group for this legume. It is necessary to use the bacteria from Wood's clover nodules for nodule production on this legume. It is quite probable that some other nodule bacteria belong to this group, but as yet they have not been found. A very large number of cross-inoculation groups undoubtedly exist and future study will add to those recognized at present.

## FIELD OBSERVATIONS

Cross-inoculation studies under controlled conditions are the only reliable procedure for determining group relationships. Under field conditions one

<sup>1</sup> Published with the permission of the Director of the Agricultural Experiment Station, Madison, Wisconsin.

may sometimes test out the controlled experiments. Bacteria-free Wood's clover seed grown in field plots where a number of common legumes had previously grown showed no inoculation. Further field seedings where vetch, lupine, alfalfa, red clover, and sanfoin soil had been applied showed no inoculation on the Wood's clover.

A field seeding will occasionally show some inoculation even with a new legume. Such observations, unless thoroughly investigated, are very likely to lead to wrong conclusions. This chance inoculation has been traced to nodule bacteria carried on the seed. Much variation has been found in different lots of seed, some lots carrying too few bacteria to be valuable for inoculation but too many for cross-inoculation studies without complete sterilization of the seed. Some yet undiscovered wild legume may be inoculating Wood's clover in occasional cases.

TABLE 1  
*Cross-inoculation experiments with Wood's clover (Dalea alopecuroides)*

KIND OF PLANT	KIND OF BACTERIA	NODULE FORMATION
Dalea	Alfalfa, strains 31, 32, 33	No nodules
Dalea	Clover	No nodules
Dalea	Pea, strains 3, 4, 10, 10A, 15, 20	No nodules
Dalea	Bean	No nodules
Dalea	Wisteria	No nodules
Dalea	Lupine	No nodules
Dalea	Locust	No nodules
Dalea	Lead Plant	No nodules
Dalea	Hog peanut	No nodules
Dalea	Cowpea	No nodules
Dalea	Soybean	No nodules
Alfalfa	Dalea	No nodules
Sweet clover	Dalea	No nodules
Dalea	Not inoculated	No nodules
Dalea	Dalea	Large abundant nodules on all plants

An example of the benefit to be derived from reinforcing any natural inoculation carried on the seed by applying inoculation appears in plate 1. Plate 2 shows the roots and nodules of Wood's clover inoculated with the culture under study.

#### NITROGEN AND PROTEIN CONTENT OF WOOD'S CLOVER

Four samples of Wood's clover of spring seeding, taken July 30 and September 4, showed an average nitrogen content of 2.66 per cent, which would equal 16.62 of protein on a water-free basis provided all the nitrogen was present in the form of protein. Six samples of plants 12 inches tall from a summer seeding averaged 4.08 per cent nitrogen or 25.80 per cent protein. Al-

though a high nitrogen content is not uncommon with young plants, this is very high for plants 54 days old. Hughes (1) has reported a protein content at the time of plowing under of 15 per cent, and, in younger plants, of 20 per cent.

#### SOME CHARACTERISTICS OF WOOD'S CLOVER NODULE BACTERIA

The root-nodule bacteria of Wood's clover were isolated from nodules taken from plants grown in the field. Purity was determined by the accepted methods, which included growth in litmus milk and inoculation on potato slopes. These organisms grew rapidly on mannitol agar. Abundant slime of a non-sticky nature was produced. Growth was opaque and resembled that of the nodule bacteria of clover more than that of alfalfa. The shape of the organisms varied with different sugars: with some they were short rods; with others large oval cells. Mannitol gave organisms  $3.3\mu$  by  $1\mu$ ; glucose,  $1.7\mu$  by  $0.83\mu$ ; sucrose, lactose, and xylose,  $1\mu$  by  $0.8\mu$ ; plain and mannitol milk,  $3-4\mu$  by  $1\mu$ . Motility was observed in mannitol and glucose solutions. The largest number of motile organisms was found with the mannitol solution. On plain agar the organisms were very motile at 24 hours. The usual irregular bacterioid shapes were seen. When stained by the Plimmer-Paine method peritrichic flagella were demonstrated.

#### GROWTH ON POTATO SLOPES

This organism shows more ability to grow on potato than most of the other known root-nodule bacteria. Cultures replated and many times transferred still show a slight watery growth when placed on potato slopes. It is not dissimilar in appearance to a heavy inoculation when the latter is observed soon after being placed on the potato. The nature of the potato slopes appears to influence the amount of growth that results. On some lots very little growth of root-nodule bacteria occurs, whereas on others much growth appears.

#### SERUM ZONE FORMATION, REACTION, AND REDUCTION IN MILKS

Growth in litmus milk gives a very deep serum zone after several weeks. Zone formation starts very rapidly as compared with other root-nodule bacteria, often showing in 2 days, and progressing to a point where it equals one-half or more of the original volume of the milk. The reaction in the serum zone is at first alkaline, then becomes neutral and later acid. The litmus becomes entirely reduced in the solid portion of the milk. Growth appears to take place at the surface and throughout the entire depth of the milk. In brom-thymol-blue milk, after several weeks a condition similar to that found in the litmus milk occurred, with the reaction in the serum zone slightly acid. In plain milk a very deep serum zone is also evident. A typical serum zone was produced in litmus milk containing 5 per cent mannitol and in one with 5 per cent sucrose, and considerably later in that to which the 5 per cent dex-



trose was added. This serum-zone formation in milk is typical of the bacillus type of root-nodule bacteria.

#### GROWTH WITH DIFFERENT SUGARS

The bacteria from this legume grew very rapidly with all the sugars except lactose. The increase in growth with that sugar was quite remarkable after a slow start—at 12 days it was equal to both sucrose and xylose. The glucose media contained peptone and beef extract, which made it not strictly comparable with the other sugars. The exceptionally rapid growth of some strains of nodule bacteria has been attributed to their being recently isolated from the plant or in recent contact with the soil. Although such an effect is often noted with some strains, with others a very great stimulation in growth results from repeated culturing, as judged on one kind of media. The *Dalea*

TABLE 2  
*Relative growths with different sugars*

SUGAR		GROWTH AFTER			
		2 days	12 days	22 days	42 days
Mannitol		++++	++++	++++	++++
Mannitol	+CaCO <sub>3</sub>	+++++	+++++	++++	++++
Sucrose		++++	++++	++++	++++
Sucrose	+CaCO <sub>3</sub>	++++	+++	+++	+++
Lactose		+	++++	+++	+++
Lactose	+CaCO <sub>3</sub>	++	+++	+++	+++++
Xylose		+++	+++	+++	++
Xylose	+CaCO <sub>3</sub>	++++	++++	+++	+++
Glucose*		+++++	+++++	++++	++++
Glucose*	+CaCO <sub>3</sub>	+++++	++++	++++	++++

\* Peptone and beef extract added.

+ indicates scant growth; ++, fair growth; +++, good growth; +++++, excellent growth; ++++++, heavy growth.

organism has so far never failed to give a very rapid growth under the conditions of this study. The strains isolated may have been fast growers only and further search may result in the isolation of slow growers.

#### CONCLUSIONS

1. Wood's clover (*Dalea alopecuroides*) does not possess root-nodule bacteria in common with any other legume group, consequently it is placed in a group by itself for inoculation purposes.

2. The nitrogen content of this annual legume is very high and compares favorably with other legumes.

3. The root-nodule organism of this legume produces in milk a serum-zone

characteristic of the bacilli group. The organism is motile and its flagella are peritrichous.

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## PLATE 1

### APPLIED INOCULATION COMPARED WITH NATURAL INOCULATION

Plants on left grown from seed inoculated with pure culture. Plants on right not inoculated; some inoculation was carried on the seed, as rows of sterilized seed showed no nodules.

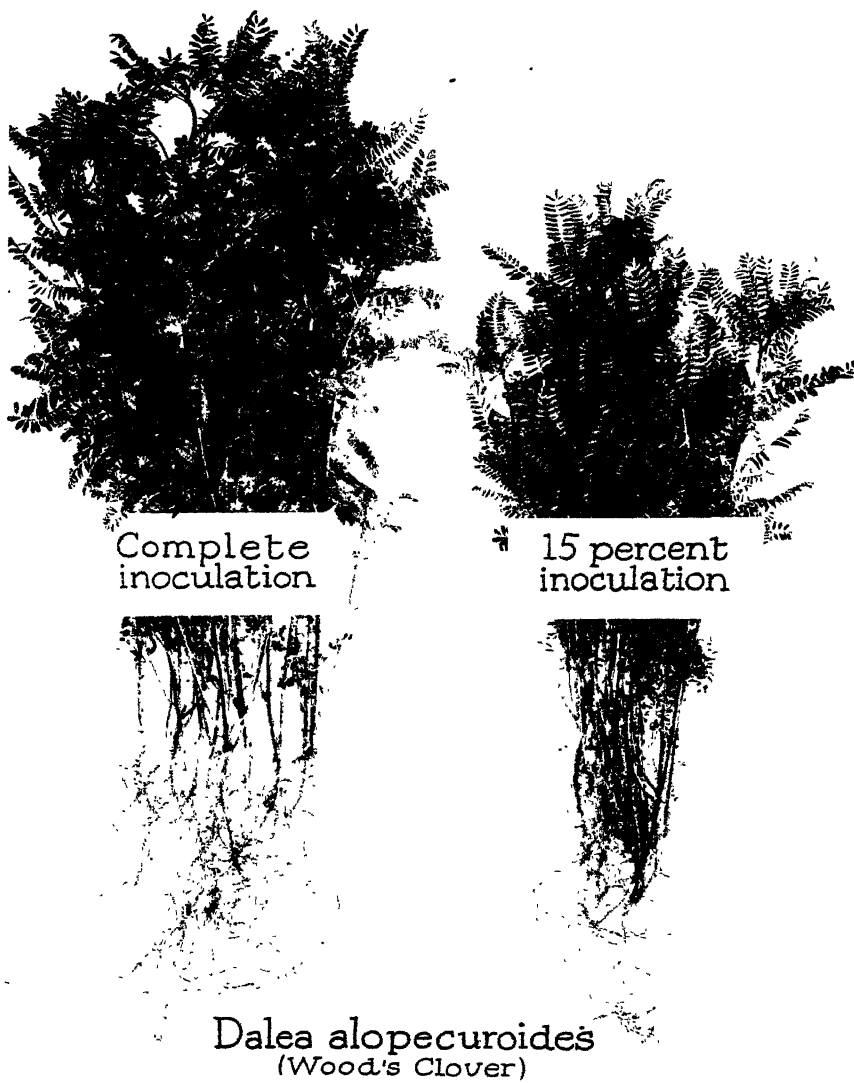


PLATE 2

FIG. 1. WOOD'S CLOVER ROOTS AND NODULES

About twice natural size

FIG. 2. WOOD'S CLOVER ROOT-NODULE BACTERIA SHOWING FLAGELLA STAINED BY  
THE PLIMMER AND PAINE METHOD

Magnification 1425. Made by Colmer



FIG. 1



FIG. 2



## THE ASSIMILATION OF PHOSPHORUS FROM PHYTIN BY OATS<sup>1</sup>

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The problem of keeping ample amounts of phosphorus in the form available for growing plants will be solved in a large measure by combining as much as possible of the total soil phosphorus into the active organic matter of the soil. The phosphorus compounds of actively decomposing organic matter are very rapidly liberated in a soluble form by soil bacteria. Organic phosphorus is not subject to conversion into iron and aluminum phosphates so readily as when applied in the inorganic forms.

The following quotation from Dr. C. M. Hutchinson (3) is of interest in this connection:

There has been much conflict of opinion amongst soil bacteriologists as to the solubilizing action of soil bacteria upon insoluble phosphates, but there can be no doubt that under certain conditions such action takes place. Work in this laboratory on this subject during the past 3 years has demonstrated conclusively the possibility of considerable and continuous solubilization of tricalcic phosphate, such as apatite, purely by bacterial action under controlled conditions in the presence of sufficient organic matter.

The rapidly increasing use of green manures offers an opportunity to take advantage of the feeding capacity of these crops by supplying them with inexpensive forms of phosphorus and thereby enabling them to build up their phosphorus content and to enhance their value for the succeeding crops. A new method of mixing inorganic phosphates with fermenting green manures is being studied in some countries. This has proved highly profitable in India. Much of the phosphorus in such a mixture would seem to be in the bacterial cells. If this is true, then another form of organic phosphorus in soil commands study. In the fermenting of high cellulose-containing materials in composts, phosphorus might be used to advantage, being converted into organic compounds by the bacteria and later being available upon their decomposition.

This opportunity to increase the content of organic phosphorus in green manures and to liberate that element from inorganic phosphates where the

<sup>1</sup> Work conducted at the University of Illinois. Submitted to the Graduate School by the junior author in partial fulfillment of the requirements for the degree of Master of Science.

Appreciation is hereby expressed for the interest and assistance given by the late Professor C. G. Hopkins.



same green manures nitrify after being plowed under, should be taken into consideration in any proper cropping system.

¶ In taking up the study of the assimilation of different forms of organic phosphorus, phytin was first chosen because of its prevalence in crop residues.

#### LITERATURE CONCERNED WITH PHYTIN

Nagaoka (4) who investigated the relative values of organic phosphates from animal and vegetable origin, found those of animal origin better for plant growth.

Aso and Yoshida (2) made comparative studies of vegetable substances containing phosphorus. Their results are given in table 1. Compared with lecithin and nucleo-protein, phytin was an intermediate source of phosphorus.

Rose (6) grew lupin seedlings in different solutions for 6 to 9 days and measured the root elongation every 3 days. As the addition of phytin did

TABLE 1  
*Comparative yields of grain with organic and inorganic phosphorus*

SUBSTANCE	HEIGHT	DRY MATTER	GRAIN
	cm.	gm.	gm.
Lecithin.....	20.4	11.7	4.5
Phytin.....	11.1	2.5	0.5
Nucleo protein.....	9.3	2.3	0.0
Tricalcium phosphate.....	21.0	9.9	3.2
Disodium phosphate.....	15.9	6.0	1.8
Iron phosphate.....	9.6	3.5	0.8
Aluminum phosphate.....	10.2	3.0	0.5
Check.....	7.8	1.4	0.0

not cause any stimulation of growth, it was thought that the acid radical might not be conducive to plant growth. The same author gave the ratio of carbon: phosphorus in phytin as 6:6. He considered phytin as more than a reserve material, and expressed the belief that, in all probability, it entered into the synthesis of phospho-proteins and lipoids.

Thompson (7) isolated phytin from rice bran and unpolished rice, but failed to obtain more than a trace from polished rice.

Patten and Hart (5) found most of the phosphorus of wheat bran to be organic and pointed out that phytin was widely distributed in the vegetable kingdom.

Anderson (1) has made the most important researches on phytin. His work includes studies on the phytin content of oats, of corn, and of wheat bran, and many papers dealing with the composition of the organic phosphorus compounds of these and other substances. It is important to note the occurrence of phytin and related inosite hexaphosphate ( $C_6H_{18}O_{24}P_6$ ) and other similar organic phosphorus compounds in farm crops.

Many data are available bearing upon the high phosphorus requirement of, and its stimulation to, soil bacteria. These references indicate the building of inorganic phosphorus into organic phosphorus by bacteria living in the soil. Such phosphorus is temporarily removed from solution but is easily returned to solution by bacterial decomposition of bacterial bodies. How much competition there may be between bacteria and higher plants for phosphorus in soils is not known. This probably would cease to be important if the nitrogen bacteria were encouraged to predominate in the soil flora.

#### EXPERIMENTAL

The pot-cultures were conducted in one-gallon jars. Clean white quartz sand that had been thoroughly leached with dilute hydrochloric acid and then washed free from acid with nitrogen-free distilled water was used. In all series except 29-56, additions of pure reprecipitated calcium carbonate and of magnesium sulfate supplied the calcium and the magnesium. Dolomite supplied both these elements for series 29-56. Kainit, free from phosphorus, was added as a potassium carrier for series 29-56, and potassium sulfate was employed for that purpose in the other series. Nitrogen was added as ammonium nitrate. The plant-food applications were made as suggested by Hopkins and Pettit.

#### PHOSPHORUS ADDITIONS

Phosphorus was supplied in the forms given in the list below.

<i>Material</i>	<i>Phosphorus per cent</i>
Phytin.....	19.20
Crude phytin.....	12.96
Alfalfa, third cutting.....	0.168
Leached alfalfa.....	0.067
Tennessee rock phosphate.....	12.120
Tricalcium phosphate.....	14.600

The phytin is termed pure phytin in this work to distinguish it from the cruder product. It was apparently a mixture of calcium phytate and phytic acids.

#### OATS SERIES 29-56

The object of this preliminary series 29-56 was to compare the yields of oats grown with organic and inorganic phosphates. Three different forms of phosphorus were used; namely, rock phosphate, pure phytin, and crude phytin. Through an error in the analysis, different amounts of phosphorus in the different forms were added. Although the treatments are not identical, they will give an idea as to the relative availability of the different forms of phosphorus. All pots excepting 29 and 30 received 28 gm. of dolomite, 1.75 gm. of kainite and soluble nitrogen in the form of ammonium nitrate. Seven kilograms

of clean quartz sand was weighed out and the dolomite, kainite, and the phosphorus-bearing substances were added and thoroughly mixed before being placed in the pots. After being wet with distilled water, a sufficient number of oat seeds was planted to insure a stand, and when the seedlings were a few days old they were thinned to ten plants per pot. According to the analysis of the oat grains, not more than 0.9 mgm. of phosphorus was added to each pot in the seed. In table 2, the treatments are expressed in milligrams of

TABLE 2  
*Treatments and yields of oats, series 29-56*

POT NUMBER	PHOSPHORUS TREATMENT PER POT	WEIGHT CROP	WEIGHT GRAIN
	mgm.	gm.	gm.
<i>Checks</i>			
29-30	None	0.8	.....
31-32	All but P	0.8	.....
<i>Rock phosphate series</i>			
33-34	212	2.7	0.015
35-36	424	3.9	0.133
37-38	848	9.8	0.118
39-40	2545	18.9	0.207
<i>Pure phytin series</i>			
41-42	221	22.9	6.743
43-44	442	18.7	4.377
45-46	883	17.8	2.375
47-48	2650	16.7	1.408
<i>Crude phytin series</i>			
49-50	175	24.2	4.598
51-52	351	25.3	5.116
53-54	702	20.4	3.197
55-56	2106	6.9	0.263

phosphorus per pot and the yields in grams per pot. All yields of the two duplicate pots are averaged in the tables.

It will be observed that the rock phosphate even in large amounts produced scarcely any grain. This may be largely due, however, to the fact that the oats matured in January. With the pure phytin, the minimum treatment produced the maximum yield. With crude phytin, the 351-mgm. treatment produced the maximum yield. The experiment tends to show two points: first, the organic phosphorus as phytin is much more readily assimilated by

plants than the phosphorus from rock phosphate; and second, phytin supplied in large amounts is deleterious as judged by the yields. By considering the amount of phosphorus supplied in the two forms of phytin it will be seen that the yields are very close together and that 220 mgm. of phosphorus per pot as phytin seemed to produce about the maximum yield. Larger amounts than this were deleterious. A toxic effect from the crude phytin was noticeable during growth.

TABLE 3  
*Percentage and total weight of phosphorus in grain and straw on oats series 29-56*

POT NUMBER	PHOSPHORUS TREATMENT PER POT	GRAIN P	STRAW P	GRAIN P	STRAW P	CROP TOTAL P
	mgm.	per cent	per cent	mgm.	mgm.	mgm.
<i>Checks</i>						
29-30	None		0.065		0.53	0.53
31-32	All but P		0.060		0.48	0.48
<i>Rock phosphate series</i>						
33-34	212	0.65	0.063	0.09	1.69	1.78
35-36	424	0.47	0.065	0.62	2.45	3.07
37-38	848	0.63	0.082	0.74	7.94	8.68
39-40	2545	0.60	0.125	1.24	23.36	24.60
<i>Pure phytin series</i>						
41-42	221	0.62	0.295	41.8	47.6	89.4
43-44	442	0.64	0.815	28.0	116.7	144.7
45-46	883	0.74	1.390	17.6	214.4	232.0
47-48	2650	0.85	1.940	11.9	296.7	308.6
<i>Crude phytin series</i>						
49-50	175	0.53	0.164	24.4	32.1	56.3
51-52	351	0.59	0.404	30.2	81.5	111.7
53-54	702	0.64	0.764	20.4	131.4	151.8
55-56	2106	0.73	0.992	1.9	65.8	67.7

Table 3 which gives the analytical results of this series, shows that there is an increase in the percentage of phosphorus for increases in phosphorus applications in both grain and straw, and that this holds for both forms of phytin and also for the rock phosphate in the grain. The grain shows only a small amount of tolerance for phosphorus, whereas the straw shows a large amount.

Plate 1 shows the growth of oats on the rock phosphate and on the crude phytin series.

Figure 1 shows graphically the percentages of phosphorus in the grain and straw.

#### OATS SERIES 101-184

This second oats series 101-184 comprising 84 pots, was much larger than the first. In this series the phosphorus was supplied in five different forms, each being added in four different amounts. In the first five series only one form of phosphorus was added to a series, whereas in the last five series, two kinds—one organic and the other inorganic—were combined in each series. The object of the first five series was mainly to check the combination series. An attempt was made to confine the applications to such amounts that pot limitation would not be a factor, but in some of the higher treatments it

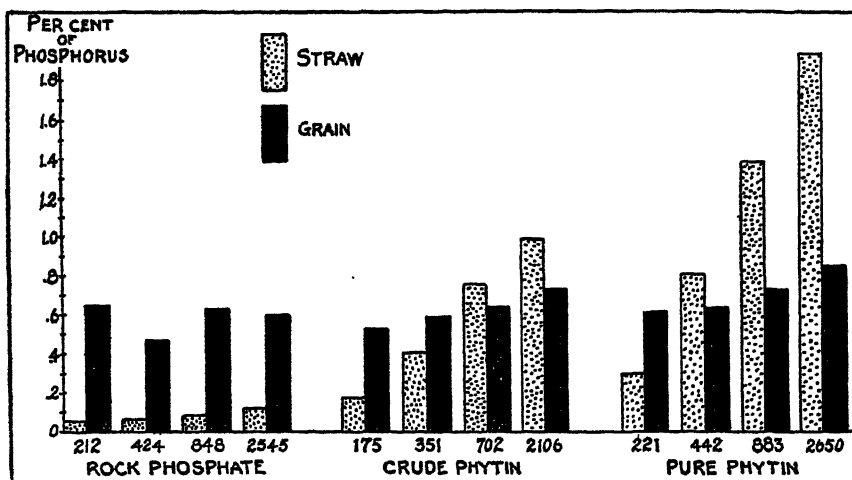


FIG. 1. COMPARATIVE PERCENTAGES OF PHOSPHORUS IN OAT STRAW AND GRAIN

The figures at the base of the double columns are the milligrams of phosphorus applied per jar.

did enter. The applications of phosphorus on the single series were made in the following forms and amounts per pot:

*Tricalcium phosphate*—28, 55, 110, and 330 mgm.

*Rock phosphate*—220, 440, 880, and 1320 mgm.

*Alfalfa*—11.9, 23.8, 35.7, and 47.6 mgm.

*Leached alfalfa*—4.9, 9.8, 14.7, and 19.6 mgm.

*Crude phytin*—11.9, 23.8, 35.7, and 47.6 mgm.

The alfalfa and leached alfalfa were applied at the rate of 7, 14, 21, and 28 gm. per pot respectively, thus adding to each pot a comparative amount of organic matter.

The combination series were made up as follows:

*Alfalfa plus rock phosphate.* To each pot of an alfalfa series was added 880 mgm. phosphorus as rock phosphate.

TABLE 4  
Treatment and weights of grain and total crop on oats series 101-144

POT NUMBER	PHOSPHORUS TREATMENT PER POT	WEIGHT CROP	WEIGHT GRAIN
	mgm.	gm.	gm.
<i>Checks</i>			
101-2	None	0.9	....
103-4	All but P	1.6	....
<i>Tricalcium phosphate series</i>			
105-6	28	2.0	0.09
107-8	55	2.0	0.12
109-10	110	3.5	0.69
111-12	330	4.6	1.17
<i>Rock phosphate series</i>			
113-14	220	7.0	1.08
115-16	440	10.3	2.15
117-18	880	15.5	3.59
119-20	1320	16.2	4.84
<i>Alfalfa series</i>			
121-22	11.9	6.6	0.96
123-24	23.8	7.0	1.73
125-26	35.7	10.5	2.26
127-28	47.6	8.5	2.48
<i>Leached alfalfa series</i>			
129-30	4.9	1.4	....
131-32	9.8	1.2	....
133-34	14.7	1.1	....
135-36	19.6	1.1	....
<i>Crude phytin series</i>			
137-38	11.9	3.7	0.48
139-40	23.8	6.3	1.09
141-42	35.7	9.5	2.04
143-44	47.6	15.7	4.07

*Leached alfalfa plus rock phosphate.* To each pot of a leached alfalfa series was added 880 mgm. of phosphorus as rock phosphate.

*Phytin plus rock phosphate.* To each pot of a phytin series was added 880 mgm. of phosphorus as rock phosphate.

*Rock phosphate plus alfalfa.* To each pot of rock phosphate series was added 21 gm. of alfalfa containing 35.7 mgm. of phosphorus.

*Rock phosphate plus phytin.* To each pot of a rock phosphate series was added 35.7 mgm. of phosphorus as phytin.

In order to allow ample time for the decomposition of the alfalfa and the establishment of an equilibrium in the sand, the fertilizers were mixed with the sand, potted, and wet down on December 28 with a liberal amount of soil infusion. On January 20, 16 kernels of oats were planted in each pot and

TABLE 5  
*Treatment and yields on combination oat series 145-184*

POT NUMBER	PHOSPHORUS TREATMENT PER POT	WEIGHT CROP	WEIGHT GRAIN	THEORETICAL YIELD
	mgm.	gm.	gm.	gm.
<i>Alfalfa plus rock phosphate</i>				
145-46	Alfalfa 11.9, R. P. 880	13.6	3.72	4.55
147-48	Alfalfa 23.8, R. P. 880	15.7	4.10	5.32
149-50	Alfalfa 35.7, R. P. 880	12.8	3.56	5.85
151-52	Alfalfa 47.6, R. P. 880	11.0	2.85	6.07
<i>Leached alfalfa plus rock phosphate</i>				
153-54	Leached alfalfa 4.9, R. P. 880	10.6	2.21	3.59
155-56	Leached alfalfa 9.8, R. P. 880	7.0	1.79	3.59
157-58	Leached alfalfa 14.7, R. P. 880	7.6	2.06	3.59
159-60	Leached alfalfa 19.6, R. P. 880	7.0	1.76	3.59
<i>Phytin plus rock phosphate</i>				
161-62	Phytin 11.9, R. P. 880	18.6	4.91	4.07
163-64	Phytin 23.8, R. P. 880	17.0	4.81	4.68
165-66	Phytin 35.7, R. P. 880	21.2	6.07	5.63
167-68	Phytin 47.6, R. P. 880	19.1	5.45	7.66
<i>Rock phosphate plus alfalfa</i>				
169-70	R. P. 220, alfalfa 35.7	12.6	3.74	3.34
171-72	R. P. 440, alfalfa 35.7	15.4	3.48	4.41
173-74	R. P. 880, alfalfa 35.7	12.8	3.56	5.85
175-76	R. P. 1320, alfalfa 35.7	15.5	4.48	7.10
<i>Rock phosphate plus phytin</i>				
177-78	R. P. 220, phytin 35.7	15.7	3.80	3.12
179-80	R. P. 440, phytin 35.7	16.0	3.88	4.19
181-82	R. P. 880, phytin 35.7	21.2	6.07	5.63
183-84	R. P. 1320, phytin 35.7	21.6	6.22	6.88

in a week there was a fine stand. In a few days most of the plants in the pots containing alfalfa or leached alfalfa had died or were severely injured. On February 4 these pots were replanted and the second time developed without any further noticeable difficulty.

Table 4 gives the treatments and yields of the oats on the singly treated series. It will be seen that for each increment of phosphorus added, there is an increase in the yield of grain until the maximum is reached. The alfalfa series produced better than the phytin in the lower treatments, but dropped off in the higher treatments.

Table 5 gives the treatments and yields of the oats on the combination series. It will be noted that in the lower treatments there is a tendency for the yields to be greater than the theoretical yields, which are obtained by taking the sum of the yields for the separate treatments in table 4.

#### SUMMARY

1. Phosphorus supplied in phytin was found to be more readily assimilated by growing plants than that in the inorganic form.
2. An increase in the rate of application increased the phosphorus content of both the grain and straw of oats, but to a greater extent of the straw. This was found to be true with both organic and inorganic phosphates.
3. A marked toleration of phosphorus was found in the straw of the oats and an indication of some tolerance of that element in the grain.
4. Large amounts of phosphorus as phytin were found to be deleterious to the growing plant.
5. Oats planted 23 days after the addition of alfalfa and leached alfalfa to sand were injured or killed, whereas those planted 14 days later in the same treatments grew normally.

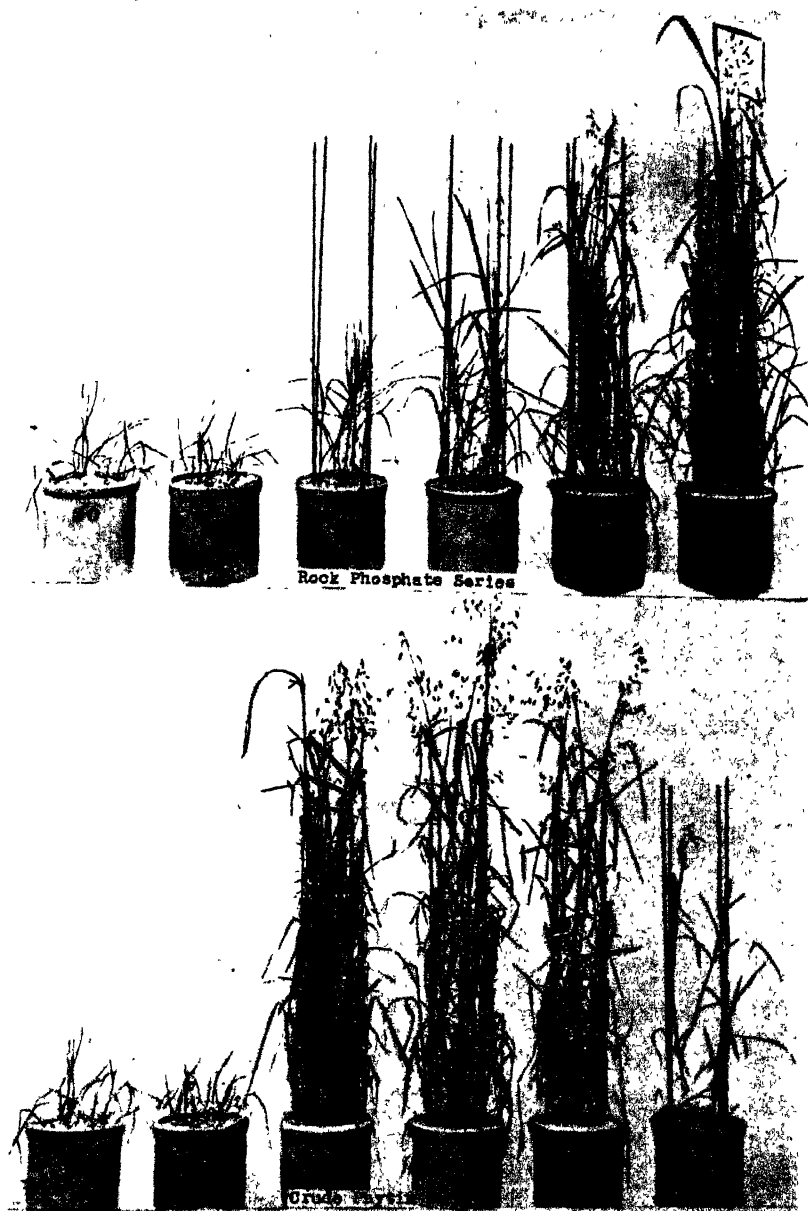
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PLATE 1

- Above:* Pot 30. No treatment  
Pot 32. All plant-food except P  
Pot 34. 212 mgm. P per pot  
Pot 36. 424 mgm. P per pot  
Pot 38. 848 mgm. P per pot  
Pot 40. 2545 mgm. P per pot
- Below:* Pot 30. No treatment  
Pot 32. All plant-food except P  
Pot 50. 175 mgm. P per pot  
Pot 52. 351 mgm. P per pot  
Pot 54. 702 mgm. P per pot  
Pot 56. 2106 mgm. P per pot



RELATIVE GROWTH OF OATS WITH ROCK PHOSPHATE AND CRUDE PHYTIN

## PLATE 2

*Above:* Pot 101. No treatment

Pot 104. All plant-food except P

Pot 114. 220 mgm. P per pot

Pot 116. 440 mgm. P per pot

Pot 118. 880 mgm. P per pot

Pot 120. 1320 mgm. P per pot

*Below:* Pot 101. No treatment

Pot 104. All plant-food except P

Pot 178. 220 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot

Pot 180. 440 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot

Pot 182. 880 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot

Pot 184. 1320 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot



### PLATE 3

*Above:* Pot 101. No treatment

Pot 104. All plant-food except P

Pot 138. 11.9 mgm. P per pot

Pot 140. 23.8 mgm. P per pot

Pot 142. 35.7 mgm. P per pot

Pot 144. 47.6 mgm. P per pot

*Below:* Pot 101. No treatment

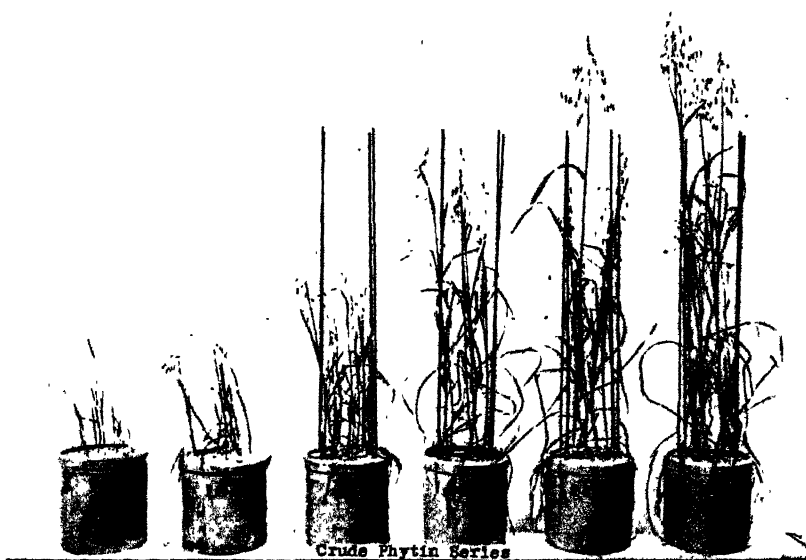
Pot 104. All plant-food except P

Pot 162. 880 mgm. P in rock phosphate + 11.9 mgm. P in phytin per pot

Pot 164. 880 mgm. P in rock phosphate + 23.8 mgm. P in phytin per pot

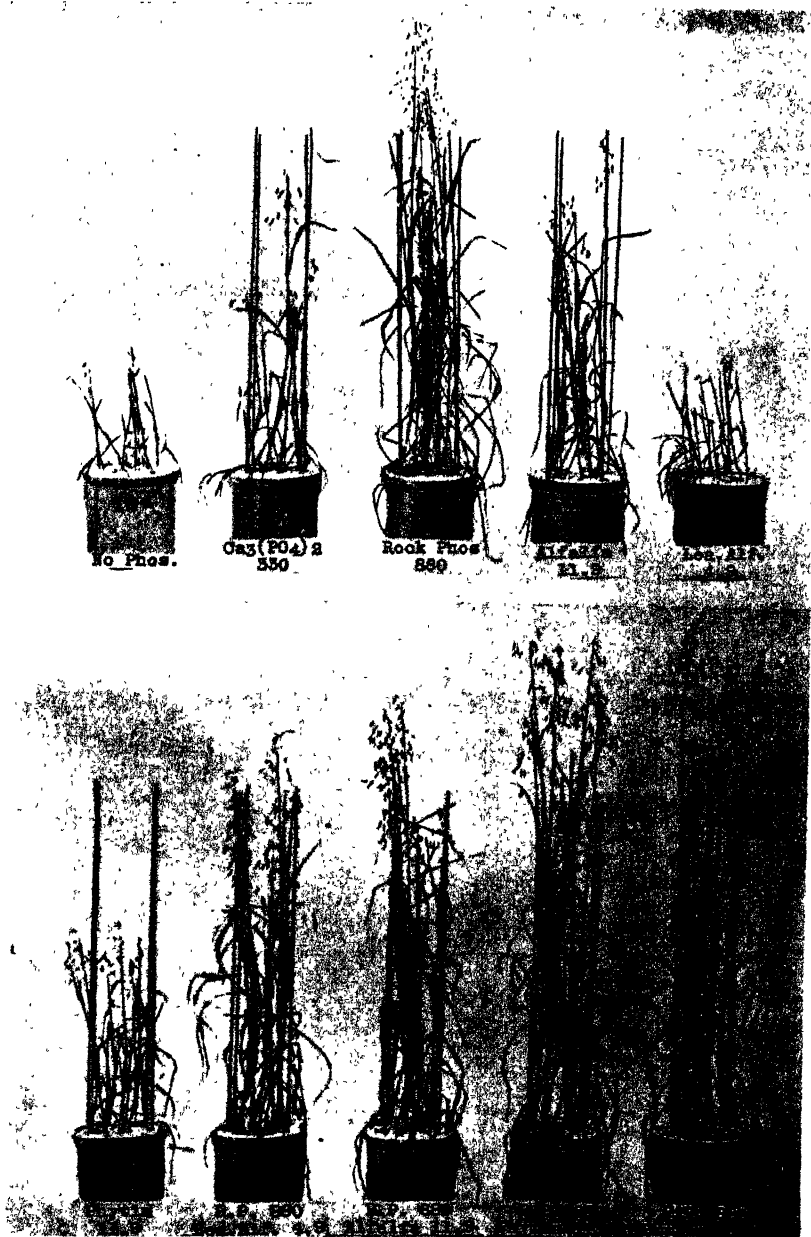
Pot 166. 880 mgm. P in rock phosphate + 35.7 mgm. P in phytin per pot

Pot 167. 880 mgm. P in rock phosphate + 47.6 mgm. P in phytin per pot



#### PLATE 4

The figures show the number of milligrams of phosphorus added in the forms indicated.



OAT SERIES WITH VARIOUS SOURCES OF PHOSPHORUS





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